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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

DATE: **December 16, 2010**

SUBJECT: **GLYPHOSATE - Report of the Endocrine Disruptor Review Team - Test Order #: EDSP – 417300- 229; 230; 240; 241; 244; 246; 247 and 248**

PC Code: 417300
Decision No.: N/A
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0055468
MRID No.: See Section V

DP Barcode: D380514 and D380541
Registration No.: N/A
Regulatory Action: N/A
Case No.: N/A
CAS No.: N/A
40 CFR: N/A

FROM: Greg Akerman, Ph.D.
Executive Secretary
Endocrine Disruptor Review Team

THROUGH: Karen Whitby, Ph.D., Co-Chair
Endocrine Disruptor Review Team
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And
Gary Timm, Co-Chair
Endocrine Disruptor Review Team
Office of Science Coordination and Policy

TO: Neil Anderson
Chemical Review Manager
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SUMMARY CONCLUSIONS

Please find below a table that summarizes the Agency's conclusions regarding the submissions provided by the Test Order Recipient and the public in response to the Agency's Test Order for the screening assays included in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery.

This table summarizes the initial response of the Test Order Recipient(s) as well as the conclusions of the Office of Chemical Safety and Pollution Prevention (OCSPP) Endocrine Disruptor Review Team (EDRT).

Chemical: Glyphosate			**PC Code: 417300**		
		Test Order Recipient Response		**Agency's Conclusions**	
Guideline	**Assay**	**Will Generate New Data**	**Existing Data Cited**	**Does Cited Data Satisfy the Order**	**Rationale**
890.1100	Amphibian Metamorphosis Assay (Frog)	No	Yes	No	See Table 1 below.
890.1150	Androgen Receptor Binding (Rat Prostate)	No	Yes	No	See Table 2 below.
890.1200	Aromatase Assay (Human Recombinant)	No	Yes	Yes	See Table 3 below.
890.1250	Estrogen Receptor Binding	No	Yes	No	See Table 4 below.
890.1300	Estrogen Receptor Transcriptional Activation (Human Cell Line HeLa-9903)	No	Yes	No	See Table 5 below.
890.1350	Fish Short-Term Reproduction	No	Yes	No	See Table 6 below.
890.1400	Hershberger (Rat)	No	Yes	No	See Table 7 below.
890.1450	Female Pubertal (Rat)	No	Yes	No	See Table 8 below.
890.1500	Male Pubertal (Rat)	No	Yes	No	See Table 9 below.
890.1550	Steroidogenesis (Human Cell Line – H295R)	No	Yes	No	See Table 10 below.
890.1600	Uterotrophic (Rat)	No	Yes	No	See Table 11 below.

N/A = Not applicable; the Test Order Recipient has agreed to conduct this assay.

The Test Order Recipient will need to conduct the Tier 1 EDSP 890 Series Guideline assays identified in the table above that the submitter agreed to perform or those that are not satisfied by the OSRI submitted.

I. BACKGROUND

On October 29, 2009, the Agency began to issue test orders for the initial list of chemicals to be tested in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery under authority provided in section 408(p)(5) of the Federal Food, Drug, and Cosmetic Act (FFDCA). The EDSP Tier 1 screening data required to satisfy an order are due within 2 years of the date of issuance of the order. The policies and procedures the Agency will use for the initial screening of chemicals are described in FRN Vol. 74, No. 71 (April 15, 2009).

The Agency formed the Endocrine Disruptor Review Team (EDRT) to support OCSPP scientists in their review of "other scientifically relevant information"[1] that may be cited by Test Order Recipients or the public in response to EDSP Tier 1 test orders. The EDRT provides a centralized venue for the review of OSRI submitted in response to the EDSP Tier 1 test orders issued under 408(p) of FFDCA to screen pesticide chemicals for their potential to interact with the estrogen; androgen and thyroid (EAT) hormonal systems. The goal of the EDRT is to reach consistent, transparent and defensible conclusions on responses to the test orders for existing data cited and submitted to the Agency which are believed to be sufficient to satisfy part or all of the EDSP Tier 1 Test Order data requirements.

II. WEIGHT OF EVIDENCE EVALUATION OF THE OSRI

Section II of this document provides a summary of the Agency review of existing data cited as OSRI by either the Test Order Recipient or the public. Existing data may include data previously submitted to the Agency in support of a registration decision believed to be relevant to one or more of the assays in the test order. The cited study and its supporting data were considered relative to the Tier 1 EDSP assay for which they were cited. The Part 158 test guideline studies cited as OSRI are listed in bibliography section (Section V) of this report[2]. The Agency conducted a weight-of-evidence determination of the significance of the data cited as OSRI by all sources (i.e., either the Test Order Recipient or the public). The synthesis of this analysis is presented in Section II which consists of eleven tables; there is a table for each of the eleven assays which comprise the Tier 1 EDSP battery. Studies evaluated by the Agency in drawing conclusions to accept or reject the OSRI rationale are presented in the table for each of the respective assays along with the Agency's rationale for the decision.

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[1] Current Working Definition: "Other scientifically relevant information" is information that informs the determination as to whether the substance may have an effect that is similar to an effect produced by a substance that interacts with the estrogen, androgen, and/or thyroid hormonal systems (e.g., information that identifies substances as having the potential to interact with the estrogen, androgen, and/or thyroid system(s); information demonstrating whether substances have an effect on the functioning of the endocrine system). OSRI may either be functionally equivalent to information obtained from the Tier 1 assays—that is, data from assays that perform the same function as EDSP Tier 1 assays—or may include data that provide information on a potential consequence or effect that could be due to effects on the estrogen, androgen or thyroid systems.

[2] The OSRI contained two citations (see Section V) that were not yet submitted to the Agency at the time of review and report. So they were not considered in this evaluation.

The EDRT's evaluations of the existing data cited in the OSRI are presented in the following tables. Each of the tables provides the citations for existing data submitted to the Agency that were considered by the EDRT in their decision making. EDRT determined whether the cited/submitted data received from the Joint Glyphosate Task Force (Test Order Recipient) and People for the Ethical Treatment of Animals (PETA) provided an accepted scientific method or protocol (and any other information relevant) to satisfy the requirements of the Test Order.

Section III of this memorandum contains a table that summarizes the endocrine-related findings in the studies cited in the submissions by the Test Order Recipient and the public that were considered in the EDRT's weight of evidence evaluation.

Section IV of this memorandum contains a table that lists studies cited in the submissions by the Test Order Recipient and the public that were not used in the EDRT's weight of evidence evaluation and provides the reasons for this decision.

Section V of this memorandum contains the bibliography of all cited data from all sources (Test Order Recipient(s) and public responses).

Table 1. Evaluation of Data Submitted in Relation to the Amphibian Metamorphosis Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1100 - Amphibian Metamorphosis Assay (Frog)

1. EDSP Assay Endpoints[1]

Study Type / Literature Citation	MRID No.	Developmental Stage	Hind Limb Length	Snout-Vent Length	Wet Body Weight	Thyroid Histopathology
Folmar *et al.* (1979)	00162296	--	--	--	--	--
Howe *et al.* (2004)	46650501	x	--	x	--	--
Mann (1999)	N/A	--	--	--	--	--
McAllister and Forbis (1978)	00108205	--	--	--	--	--
Takacs *et al.* (2002)	N/A	--	--	--	--	--
Trotter (1990)	N/A	--	--	--	--	--
Xie (2005)	N/A	--	--	--	--	--
Avian Reproduction – Mallard Duck	00111953	--	--	--	--	--
Avian Reproduction – Bobwhite Quail	00108207	--	--	--	--	--
Eight-day Dietary LC50 – Bobwhite Quail	00076492	--	--	--	--	--
Eight-day Dietary LC50 – Mallard Duck	00108107	--	--	--	--	--
Full Life Cycle Study in Fathead Minnows	00108171	--	--	--	--	--
Carcinogenicity – Mouse	00130406	--	--	--	--	x
Chronic Toxicity/Carcinogenicity – Rat (1981)	00093879	--	--	--	--	x
Chronic Toxicity/Carcinogenicity – Rat (1990)	41643801	--	--	--	--	x
Chronic Toxicity – Dog	00153374	--	--	--	-	x
Subchronic – Rat	40559401	--	--	--	--	x
Subchronic – Rat (NTP, 1992)	N/A	--	--	--	--	x
Subchronic – Mouse (NTP, 1992)	N/A	--	--	--	--	x
Subchronic – Mouse	00036803	--	--	--	--	x
Three-Generation Reproduction – Rat (1981)	00081674	--	--	--	--	--
Two-Generation Reproduction – Rat (1990)	41621501	--	--	--	--	--

Table 1. Evaluation of Data Submitted in Relation to the Amphibian Metamorphosis Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1100 - Amphibian Metamorphosis Assay (Frog)

2. Summary of Study Findings:

Study Type / Literature Citation	MRID No.	Findings
Folmar *et al.* (1979)	00162296	See Section IV of the report.
Howe *et al.* (2004)	46650501	See discussion below in Section 3.
Mann (1999)	N/A	See Section IV of the report.
McAllister and Forbis (1978)	00108205	See Section IV of the report.
Takacs *et al.* (2002)	N/A	See Section IV of the report.
Trotter (1990)	N/A	See Section IV of the report.
Xie (2005)	N/A	See discussion below in Section 3.
Avian Reproduction – Mallard Duck	00111953	No treatment-related effects on endpoints related to reproduction, growth, or survival were seen at any dietary concentration tested (up to 1000 ppm).
Avian Reproduction – Bobwhite Quail	00108207	No treatment-related effects on endpoints related to reproduction, growth, or survival were seen at any dietary concentration tested (up to 1000 ppm).
Eight-day Dietary LC50 – Bobwhite Quail	00076492	See Section IV of the report.
Eight-day Dietary LC50 – Mallard Duck	00108107	See Section IV of the report.
Full Life Cycle Study in Fathead Minnows	00108171	No treatment-related effects on endpoints related to reproduction, growth or survival were seen at any concentration level tested (up to 25.7 mg/L).
Carcinogenicity – Mouse	00130406	Thyroid weights were not measured. No treatment-related histopathological lesions were seen in the thyroid, adrenal and pituitary glands.
Chronic Toxicity/Carcinogenicity – Rat (1981)	00093879	Thyroid C-cell carcinomas were non-significantly increased at the high dose (11%) compared to controls (2%). No increases were seen in adenomas and the incidences of C-cell hyperplasia were comparable between the treated and the control groups.
Chronic Toxicity/Carcinogenicity – Rat (1990)	41643801	The incidences of thyroid C-cell adenomas were increased in males at the mid (13.8%) and high dose (11.7%) groups and in females at the mid (10%)

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890.1100 - Amphibian Metamorphosis Assay (Frog)		
		and high (10%) dose groups compared to control males (3.3%) and females (3.3%). These increases were not considered to be treatment-related because: 1) none of the increases reached statistical significance; 2) absence of dose-response; 3) no increase in severity of grade or incidence in hyperplasia; 4) lack of progression to malignancy; and 4) the incidences were within the testing laboratories historical control ranges.
Chronic Toxicity – Dog	00153374	No treatment-related changes were seen in absolute or relative thyroid weights nor were there any histopathological lesions of the thyroid glands.
Subchronic – Rat	40559401	Thyroid weights were not measured. No treatment-related histopathological lesions were seen in the thyroid, adrenal and pituitary glands.
Subchronic – Rat (NTP, 1992)	N/A	Thyroid weights were not measured. No treatment-related histopathological lesions were seen in the thyroid, adrenal and pituitary glands.
Subchronic – Mouse (NTP, 1992)	N/A	Thyroid weights were not measured. No treatment-related histopathological lesions were seen in the thyroid, adrenal and pituitary glands.
Subchronic – Mouse	00036803	Thyroid weights were not measured. No treatment-related histopathological lesions were seen in the thyroid, adrenal and pituitary glands.
Three-Generation Reproduction – Rat (1981)	00081674	No treatment-related effects were observed on mating, pregnancy, and fertility indices or fetal sex ratio for either sex over the three generations. No treatment-related histopathological lesions were seen in the thyroid, pituitary and adrenal gland of the F0, F1 and F2 adults or the F3b offspring.
Two-Generation Reproduction – Rat (1990)	41621501	No treatment-related effects were observed in the reproductive performance (male or female mating indices, male or female fertility indices, gestation index, gestation length, live birth index, viability index, lactation index, parturition, or fetal sex ratio). Thyroid weight and thyroid histopathology were not evaluated.

Table 1. Evaluation of Data Submitted in Relation to the Amphibian Metamorphosis Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1100 - Amphibian Metamorphosis Assay (Frog)

3. Agency's Evaluation of the OSRI:

The submission provided by the Test Order Recipient states that "The amphibian metamorphosis assay has been included in the Tier 1 battery as a generalized model for impacts on vertebrate thyroid functions because the amphibian thyroid system is regulated and responds in a similar manner to perturbation as that of other tetrapods, including mammals (Forte *et al.*., 2007). Therefore, combined results from existing glyphosate studies provide information that is functionally equivalent to that generated in the amphibian metamorphosis assay. Thyroid histopathology has been evaluated in a three-generation reproduction study (Schroeder and Hogan, 1981), a 90-day subchronic study (Stout and Johnson, 1987) and a chronic rat study (Stout and Rucker, 1990), which did not identify any glyphosate treatment-related effects to the thyroid glands.

Confirmatory information was developed for an aquatic vertebrate in the fish full life-cycle study that shows no impact on growth and development, which are sensitive measurement endpoints for impacts on thyroid structure and function. Additionally, information of no measurable effects on thyroid function can be evaluated with data from avian reproduction studies. No effects on growth were observed in four avian reproduction studies that tested glyphosate dietary concentrations greater than 1000 ppm. Given all of this available information on glyphosate, the amphibian metamorphosis screening assay does not offer any new relevant information to the toxicology data set for Tier 1 screening purposes."

The submission by PETA states that "Tables 2 and 3 summarize available amphibian and fish data. Glyphosate active (GA) is much less toxic than Roundup® to both fish and amphibians, and there is no evidence of endocrine activity.

When comparing glyphosate active with a commercially available formula (e.g. Round-Up), researchers show that there is a significant difference in toxicity. When exposed to GA, *Oncorhynchus mykiss* (rainbow trout) had a 96-h LC50 range of 148-211 mg/L whereas the Roundup formulation was 14 mg/L (Takacs 2002).

Three tests on fish species, one bluegill and two with fathead minnow, showed LC50s of 120 ppm, 84.9 ppm, and 97 ppm, respectively. Two rainbow trout 96-hour LC50 tests provided values of 86 ppm and140 ppm. Based on these tests, glyphosate active ranges from slightly to practically non-toxic to freshwater fish species (McAllister and Forbis 1978, ID #234395; EG & G Bionomics 1975, ID #00108171 and Folmar, Sanders, and Julin 1979, ID #249160). Juvenile Rainbow trout (sex not specified) were exposed to 0.11 mg/L glyphosate active. Glyphosate did not induce elevated levels of vitellogenin in juvenile rainbow trout compared with control fish (Xie

Table 1. Evaluation of Data Submitted in Relation to the Amphibian Metamorphosis Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1100 - Amphibian Metamorphosis Assay (Frog)	

2005)."

On review of the OSRI submitted, the Agency noted deficiencies and therefore has outstanding questions about the potential for glyphosate to interact with the HPT axis:

- A lack of thyroid effects in mammalian *in vivo* studies cited does not necessarily demonstrate the absence of interaction with the thyroid. The complexity of the thyroid axis in amphibians yields many different possible mechanisms of inhibiting metamorphic processes at differing biochemical and molecular levels. Thus, changes seen in metamorphosis from interference with the thyroid axis, especially those involving effects in peripheral tissues, are pronounced and may not be apparent in existing mammalian assays.

- Although the avian reproduction studies with mallard duck and bobwhite quail and the fish full life cycle study with fathead minnow included measurements of growth, which may be an indicator of thyroid function, they lack endpoints specific to thyroid function, *e.g.*, thyroid weights, histopathology and/or hormone measurements.

- Howe *et al.* (2004) exposed *Rana pipiens* tadpoles to 0.6 and 1.8 mg acid equivalents/L glyphosate technical (isopropylamine salt) for 42 days (followed by rearing in clean water) from Gosner stage 25 until metamorphic climax (Gosner stage 42) and assessed mortality, snout-vent length, total length, body length, tail length, maximum tail height, visible damage to tail, sex ratio, gonadal development, and thyroid hormone receptor (TR) mRNA. Although this study assessed developmental parameters (e.g., time to metamorphosis, snout-vent length), it did not include measurements on tissues associated specifically with the thyroid axis/activity (e.g., thyroid histopathology, hind leg length) besides TR mRNA.

- Xie *et al.* (2005) was not considered towards satisfying the requirement for the Amphibian Metamorphosis Assay because it measured the estrogenic activity of glyphosate using a rainbow trout vitellogenin assay.

Conclusion: Based on the deficiencies and unanswered questions listed above, the data cited as OSRI did not satisfy the requirement for the Amphibian Metamorphosis Assay using Guideline 890.1100.

[1] -- = not measured; X = indicates the endpoint was measured in the assay but does not indicate whether or not it satisfies the data requirement of the test order. See section 3 below for a detailed explanation; N/A = not applicable

Table 2. Evaluation of Data Submitted in Relation to the Androgen Receptor Binding Assay

Chemical: Glyphosate	**PC Code: 417300**

890.1150 - Androgen Receptor Binding Assay (Rat Prostate)

1. EDSP Assay Endpoints[1]

Study Type / Literature Citation	**MRID No.**	**Binding Curve fit to Hills four-parameters are:**			
		Top	**Bottom**	**Slope**	**Log (IC_{50})**
Gasnier *et al.* (2009)	N/A	--	--	--	--
Kojima *et al.* (2004)	48033008	--	--	--	--
NTP, (1992) Subchronic – Rat	N/A	--	--	--	--
Morrissey (1988)	N/A	--	--	--	--
Petit *et al.* (1997)	N/A	--	--	--	--
Richard *et al.* (2005)	N/A	--	--	--	--
Romano *et al.* (2010)	N/A	--	--	--	--
Takacas *et al.* (2002)	N/A	--	--	--	--
USEPA (1998a)	N/A	--	--	--	--
Walsh *et al.* (2000)	N/A	--	--	--	--
Williams *et al.* (2000)	N/A	--	--	--	--

2. Summary of Study Findings:

Study Type / Literature Citation	**MRID No.**	**Findings**
Gasnier *et al.* (2009)	N/A	See discussion below in Section 3.
Kojima *et al.* (2004)	48033008	See discussion below in Section 3.
NTP, (1992) Subchronic – Rat	N/A	See discussion below in Section 3.
Morrissey (1988)	N/A	See discussion below in Section 3.
Petit *et al.* (1997)	N/A	See discussion below in Section 3.
Richard *et al.* (2005)	N/A	See discussion below in Section 3.
Romano *et al.* (2010)	N/A	See Section IV of this report.
Takacas *et al.* (2002)	N/A	See Section IV of this report.

Table 2. Evaluation of Data Submitted in Relation to the Androgen Receptor Binding Assay		
Chemical: Glyphosate		**PC Code: 417300**
890.1150 - Androgen Receptor Binding Assay (Rat Prostate)		
USEPA (1998a)	N/A	See Section IV of this report.
Walsh *et al.* (2000)	N/A	See Section IV of this report.
Williams *et al.* (2000)	N/A	See Section IV of this report.

3. Agency's Evaluation of the OSRI:

The submission provided by the Test Order Recipient states that "Data generated by Kojima *et al.*, (2004) clearly and consistently demonstrate that glyphosate is not an androgen receptor agonist or antagonist. This peer-reviewed article describes an *in vitro* test system and methodology that is comparable and functionally equivalent to the methodology for the AR binding assay in the Tier I screening battery described in OPPTS Guideline 890.1150. The rationale for functional equivalence is provided below.

- Specifically, Kojima *et al.* evaluated the ability of glyphosate to agonize AR binding or antagonize radio labeled [^{3}H]-R188 binding to the AR, which is the sole purpose of the AR competitive radioligand binding assay.

- The methodology of Kojima *et al.* was capable of not only detecting AR agonists and antagonists but also to differentiate between AR agonists and antagonists. The AR competitive radio ligand binding assay is not capable of making this differentiation.

- Overall, IC_{50} values with androgenic compounds for AR binding and transactivation assays are comparable to the IC_{50} values for the OPPTS 890.1150 AR competitive binding assay. This is plausible and not unexpected since both assays are measuring the same functional endpoint, AR-ligand binding.

- The transactivation assay, which has already been conducted on glyphosate, provides data of the same nature and quality as the competitive binding assay.

This finding of no androgen binding potential is further supported by a structural activity relationship analysis that indicates that glyphosate does not have androgenic potential. Additionally, comprehensive *in vivo* data obtained from chronic studies with glyphosate demonstrate no effects on endpoints that are sensitive and specific to compounds that have androgenic or anti-androgenic activity. A detailed review of the *in vivo* data that confirms the *in vitro* data that shows that glyphosate is not androgenic or anti-androgenic is extensively reviewed in the sections on the Hershberger, male pubertal and fish screening assays presented later in this document.

Table 2. Evaluation of Data Submitted in Relation to the Androgen Receptor Binding Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1150 - Androgen Receptor Binding Assay (Rat Prostate)

The data generated by Kojima *et al.* (2004), which demonstrates that glyphosate is not an androgen receptor agonist or antagonist, is substantially equivalent to the Androgen Receptor Binding Assay (OPPTS 890.1150) defined in the EDSP Tier I test battery and no additional *in vitro* screening is needed or should be required to evaluate glyphosate's potential for androgen receptor agonism and antagonism."

The submission from PETA states that: "Using a human hepatic cell line (HepG2), researchers measured cytotoxicity using three aromatase assays (Alamar Blue®, MTT, ToxiLight®), plus genotoxicity (comet assay), anti-estrogenic and antiandrogenic effects using gene reporter tests." "GA alone had *no anti-estrogenic activity* but did have a *slight anti-androgenic effect in vitro* (Gasnier 2009)."

"In a subchronic toxicity study conducted in rats by NTP (1992), reduced epididymal sperm concentrations (20% below control) were reported in F344 rats at both the 1638 mg/kg (25,000 ppm) and the 3393 mg/kg (50,000 ppm) levels. Nevertheless, all values were well within the normal range of sperm concentration values reported by the NTP in an analysis of their historical control data for these rodents (Morrissey *et al.*, 1988). As the apparent reductions were not related to dosage or accompanied by decreases in epididymal weights or testicular sperm numbers/weight, the relationship to treatment is doubtful. Moreover, ***male fertility was not reduced*** in the reproduction study even at the highest dietary level tested (30,000 ppm) (NTP 1992).

In a ***pubertal*** study, male weanling Wistar rats were dosed at 5, 50, or 250mg/kg of Roundup®. Body weight was not affected although a significant delay in puberty, significant weight decrease of adrenals, and a slight decrease in testosterone levels was seen in all three treatment groups. No pathological altercations of adrenals were seen and corticosterone and estradiol levels were not different. Researchers concluded that the direct action of the active ingredient and was not likely the major cause of the puberty delay and that glyphosate active did not present harmful effects on fertility, but instead showed effects for its adjuvant components. (Romano 2010).

The data indicate that GA does not affect male fertility or hormone levels."

After reviewing the existing data for both GA and commercially available formulations, there *is no evidence that the active ingredient causes endocrine disruption*. The mechanism of glyphosate activity is not related to endocrine pathways and should not modulate any endocrine activity and experimental results support this hypothesis. Immune response depression and sperm content reduction are endpoints which could potentially be connected with endocrine effects are also not shown to be caused *in vitro* and *in vivo* by glyphosate (Takacas 2002).

Table 2. Evaluation of Data Submitted in Relation to the Androgen Receptor Binding Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1150 - Androgen Receptor Binding Assay (Rat Prostate)

No effects were observed in numerous, multigeneration reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and its primary degradate AMPA are *not reproductive toxicants* and *do not perturb the endocrine system.*

The U.S. EPA (1998a) reviewed these studies and also concluded that there was *no evidence to suggest that glyphosate produces endocrine-modulating effects*. The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies.

The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies (Williams 2000).

Data is plentiful and well balanced, including many *in vitro* and *in vivo* studies, covering identical endpoints indicated in the EDSP. *No additional data is needed* to screen glyphosate active for endocrine disrupting activity because the data taken together clearly indicate no effects."

On review of the OSRI submitted, the Agency noted a number of deficiencies and therefore has outstanding questions about the potential for glyphosate to interact with androgen receptor.

- Gasnier *et al.* (2009) – tested glyphosate (and several product formulations containing glyphosate) for anti-androgenic activity using MDA-kb2 cells in a transcriptional activation assay. Glyphosate was shown to be anti-androgenic. In general, transcriptional activation assays and receptor binding assays are both needed to increase confidence in minimizing false negatives. Transcriptional activation assays do not measure receptor binding and involve post-binding events; therefore, they are not an adequate substitute for receptor binding assays.

- Kojima *et al.* (2004) tested glyphosate in transcriptional activation assays for androgenicity and anti-androgenicity using CHO-K1 cells. Negative results were reported, but no data were provided. In general, transcriptional activation assays and receptor binding assays are both needed to increase confidence in minimizing false negatives. Transcriptional activation assays do not measure

Table 2. Evaluation of Data Submitted in Relation to the Androgen Receptor Binding Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1150 - Androgen Receptor Binding Assay (Rat Prostate)	

receptor binding and involve post-binding events; therefore, they are not an adequate substitute for receptor binding assays.

- Morrissey (1988) is a NTP study that looked at sperm morphology and vaginal cytology on a series of chemicals. Data specific to glyphosate was not reported in this article. (See Section IV of this report).
- The NTP (1992) subchronic toxicity study in rats did not measure binding of the chemical to the androgen receptor.
- Petit *et al.* (1997) evaluated glyphosate in a reporter assay using recombinant yeast cells expressing trout estrogen receptor. This paper did not evaluate binding to the androgen receptor.
- Richard *et al.* (2005) performed assays for aromatase activity and did not measure binding to the androgen receptor.
- Romano *et al.* (2010 see Section IV of this report.
- Takacas (2002) see Section IV of this report.
- USEPA (1998a) see Section IV of this report
- Walsh *et al.* (2000) See Section IV of this report.
- Williams *et al.* (2000) see Section IV of this report.

4. Conclusion:

Based on the deficiencies and outstanding questions discussed above, the data cited as OSRI did not satisfy the requirement for the Androgen Receptor Binding Assay using Guideline 890.1150.

[1] -- = not measured; X = indicates the endpoint was measured in the assay but does not indicate whether or not it satisfies the data requirement of the test order. See section 3 below for a detailed explanation; N/A = not applicable

Table 3. Evaluation of Data Submitted in Relation to the Aromatase (Human Recombinant) Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1200 - Aromatase Assay (Human Recombinant)

1. EDSP Assay Endpoints[1]

Study Type / Literature Citation	MRID No.	^3H_2O measured	Estrone measured
Gasnier *et al.* (2009)	N/A	x	--
Richard *et al.* (2005)	N/A	x	--
Romano *et al.* (2010)	N/A	--	--
Takacas *et al.* (2002)	N/A	--	--
USEPA (1998a)	N/A	--	--
Walsh *et al.* (2000)	N/A	--	--
Williams *et al.* (2000)	N/A	--	--
1981: Three-Generation Reproduction- Rat	00081674	--	--
1990: Two-Generation Reproduction – Rat	41621501	--	--
Developmental Toxicity – Rat	00046362	--	--
Developmental Toxicity – Rabbit	00046363	--	--
1981: Chronic Toxicity/Carcinogenicity – Rat	00093879	--	--
1990: Chronic Toxicity/Carcinogenicity – Rat	41643801	--	--
Carcinogenicity – Mouse	00130406	--	--
Chronic Toxicity – Dog	00153374	--	--
Subchronic – Rat	40559401	--	--
Subchronic – Rat (NTP, 1992)	N/A	--	--
Subchronic – Mouse (NTP, 1992)	N/A	--	--
Subchronic – Mouse	00036803	--	--

2. Summary of Study Findings:

Study Type / Literature Citation	MRID No.	Findings
Gasnier *et al.* (2009)	N/A	See discussion below in Section 3.

Table 3. Evaluation of Data Submitted in Relation to the Aromatase (Human Recombinant) Assay		
Chemical: Glyphosate		**PC Code: 417300**
890.1200 - Aromatase Assay (Human Recombinant)		
Richard *et al.* (2005)	N/A	See discussion below in Section 3.
Romano *et al.* (2010)	N/A	See Section IV of this report.
Takacas *et al.* (2002)	N/A	See Section IV of this report.
USEPA (1998a)	N/A	See Section IV of this report.
Walsh *et al.* (2000)	N/A	See Section IV of this report.
Williams *et al.* (2000)	N/A	See Section IV of this report.
Part 158 studies cited above	See Above	The cited *in vivo* Part 158 mammalian toxicity studies do not measure aromatase activity.

3. Agency's Evaluation of the OSRI:

The submission provided by the Test Order Recipient states that "Although *in vitro* data is not available to assess aromatase inhibition potential of glyphosate, other scientifically relevant information of an equivalent nature and unquestionable quality is available from several existing *in vivo* chronic studies. A number of male and female endpoints (pathologies) in the two generation rat studies are predictive of aromatase inhibition and considered to be functionally equivalent to the data developed in the *in vitro* assay in the Tier I battery of the EDSP. These endpoints include:

- testicular/uterine/ovary weights and histopathology
- the size, function and histopathology of sex organs
- anogenital distance
- sperm count and mobility
- ovarian evaluation for follicles and corpa lutea
- age and weight at preputial separation and vaginal opening
- number of implantation sites and lactation

No treatment related effects were observed in these studies on any of these sensitive and specific endpoints that would be indicative of aromatase inhibition and provides strong and consistent evidence that glyphosate is not an inhibitor of aromatase activity. Information on the potential effect of glyphosate on steroidogenesis, which is also indicative of aromatase inhibition, is comprehensively discussed in the next section. The scientific information presented here from existing standard regulatory *in vivo* studies should be considered as unequivocal proof that glyphosate does not inhibit aromatase activity in the male or female, and, consequently, the requirement for performing the *in vitro* aromatase assay (OPPTS 890.1200) of the EDSP should be waived."

Table 3. Evaluation of Data Submitted in Relation to the Aromatase (Human Recombinant) Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1200 - Aromatase Assay (Human Recombinant)

The submission from PETA states that "Using a human hepatic cell line (HepG2), researchers measured cytotoxicity using three aromatase assays (Alamar Blue®, MTT, ToxiLight®), plus genotoxicity (comet assay), anti-estrogenic and antiandrogenic effects using gene reporter tests. Liver cells are sensitive to toxins as they function in detoxification *in vivo*. Results confirmed that the nature of the adjuvant in commercially available formulations has a greater affect on toxicity than the amount of glyphosate active. *Aromatase activity was not affected* by GA alone. The *in vitro* responses were dependent on formulation variations and not dose dependent with regard to GA concentrations. GA alone had *no anti-estrogenic activity* but did have a *slight anti-androgenic effect in vitro* (Gasnier 2009).

In another *in vitro* study, glyphosate active and Roundup® were applied to human placental JEG3 cells to determine endocrine activity, specifically on aromatase. Cytotoxicity increased with time (8-fold at 0.8%between 24 and 48 hr), and the median lethal dose (LD50) was approximately 1.8 times lower for Roundup® (0.7%) than for glyphosate active. After 1 hr of incubation, estrogen synthesis was enhanced by about 40% with Roundup but not with glyphosate active. After 18 hr of incubation, aromatase activity *in vitro* was inhibited, again with Roundup® only. This inhibition of aromatase activity is assumed to be an effect on aromatase gene expression, attributed to the adjuvant and not GA (Richard 2005)."

"The data indicate no effect *in vitro* on aromatase and estrogen *with perhaps a slight anti-androgenic effect for* GA. Adjuvants used in commercial formulations, however, may affect endocrine activity."

"After reviewing the existing data for both GA and commercially available formulations, there *is no evidence that the active ingredient causes endocrine disruption.* The mechanism of glyphosate activity is not related to endocrine pathways and should not modulate any endocrine activity and experimental results support this hypothesis. Immune response depression and sperm content reduction are endpoints which could potentially be connected with endocrine effects are also not shown to be caused *in vitro* and *in vivo* by glyphosate (Takacas 2002).

No effects were observed in numerous, multigeneration reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and its primary degradate AMPA are *not reproductive toxicants* and *do not perturb the endocrine system.*

Table 3. Evaluation of Data Submitted in Relation to the Aromatase (Human Recombinant) Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1200 - Aromatase Assay (Human Recombinant)	

The U.S. EPA (1998a) reviewed these studies and also concluded that there was *no evidence to suggest that glyphosate produces endocrine-modulating effects*. The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies. The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies (Williams 2000).

Data is plentiful and well balanced, including many *in vitro* and *in vivo* studies, covering identical endpoints indicated in the EDSP. *No additional data is needed* to screen glyphosate active for endocrine disrupting activity because the data taken together clearly indicate no effects."

On review of the OSRI submitted, the Agency noted the following:

- Gasnier *et al.* (2009) measured aromatase activity (using the tritiated water detection method) and mRNA induction in HepG2 cells exposed to glyphosate and various product formulations. Glysophate did not inhibit aromatase activity in this test system.
- Richard *et al.* (2005) studied glyphosate and several product formulations in JEG-1 cells using radioimmunoassay (RIA) as the method of detecting reaction products. Induction of mRNA was also followed and aromatase activity was also assessed using the placental microsomal assay by measuring the formation of tritiated water. Aromatase was inhibited by glyphosate. This study provides adequate information to satisfy the test order.
- Romano *et al.* (2010) see Section IV of this report.
- Takacas (2002) see Section IV of this report.
- USEPA (1998a) see Section IV of this report.
- Walsh *et al* (2000) see Section IV of this report.

Table 3. Evaluation of Data Submitted in Relation to the Aromatase (Human Recombinant) Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1200 - Aromatase Assay (Human Recombinant)	
• Williams *et al.* (2000) see Section IV of this report. • None of the cited Part 158 studies measure aromatase activity, which is the information that would be obtained by the Tier 1 Aromatase Assay. The argument presented in the explanations submitted to the Agency was that no aromatase-mediated effects were seen in any of the cited studies. The available data do not permit the Agency to establish confident linkages between effects on apical endpoints measured in whole animal studies and inhibition of aromatase enzyme activity.	
4. Conclusion: The requirement for the **Aromatase Assay (890.1200) is satisfied** based on the Richard *et al.* (2005).	

[1] -- = not measured; X = indicates the endpoint was measured in the assay but does not indicate whether or not it satisfies the data requirement of the test order. See section 3 below for a detailed explanation; N/A = not applicable

Table 4. Evaluation of Data Submitted in Relation to the Estrogen Receptor Binding Assay

Chemical: Glyphosate	**PC Code: 417300**

890.1250 - Estrogen Receptor Binding Assay

1. EDSP Assay Endpoints[1]

Study Type / Literature Citation	**MRID No.**	**Binding Curve fit to Hills four-parameters are:**			
		Top	**Bottom**	**Slope**	**Log(IC_{50})**
Gasnier *et al.* (2009)	N/A	--	--	--	--
Kojima *et al.* (2004)	48033008	--	--	--	--
Morrissey *et al.* (1988)	N/A	--	--	--	--
NTP, (1992) Subchronic – Rat	N/A	--	--	--	--
Petit *et al.* (1997)	N/A	--	--	--	--
Takacas *et al.* (2002)	N/A	--	--	--	--
USEPA (1998a)	N/A				
Walsh *et al.* (2000)	N/A	--	--	--	--
Williams *et al.* (2000)	N/A	--	--	--	--
Xie (2005)	N/A	--	--	--	--

2. Summary of Study Findings:

Study Type / Literature Citation	**MRID No.**	**Findings**
Gasnier *et al.* (2009)	N/A	See discussion below in Section 3.
Kojima *et al.* (2004)	48033008	See discussion below in Section 3.
Morrissey *et al.* (1988)	N/A	See Section IV of this report.
NTP, (1992) Subchronic – Rat	N/A	See discussion below in Section 3.
Petit *et al.* (1997)	N/A	See discussion below in Section 3.
Takacas *et al.* (2002)	N/A	See Section IV of this report.
USEPA (1998a)	N/A	See Section IV of this report.
Walsh *et al.* (2000)	N/A	See Section IV of this report.
Williams *et al.* (2000)	N/A	See Section IV of this report.
Xie (2005)	N/A	See discussion below in Section 3.

Table 4. Evaluation of Data Submitted in Relation to the Estrogen Receptor Binding Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1250 - Estrogen Receptor Binding Assay

3. Agency's Evaluation of the OSRI:

The submission provided by the Test Order Recipient states that "Data generated by Kojima *et al.*, (2004), Petit *et al.* (1997) and Xie (2005) in peer-reviewed journals clearly and consistently demonstrate that glyphosate does not have an ER binding potential, either as an agonist or antagonist. The methodologies employed by Kojima and Petit in these *in vitro* test systems are comparable to the overall approach of the two Tier I ER binding assays of the EDSP- the ERα/β competitive radio ligand binding assay using rat uterine cytosol (OPPTS 890.1250) and the ERα transactivation assay (OPPTS 890.1300). Specifically, transcriptional activation assays performed by Kojima *et al.* (2004) are functionally equivalent to the ERα/β competitive radio ligand binding assay (OPPTS 890.1250) and the ERβ transactivation assay (OPPTS 890.1300) for the following reasons:

- Kojima *et al.* (2004) evaluated the ability of glyphosate to agonize and antagonize ERα and ERβ binding, which is the purpose of the ERα/β competitive radio ligand binding assay (OPPTS 890.1250). The methodology of Kojima *et al.* was capable of differentiating between ERα/β agonists and antagonists, which the ERα/β competitive radio ligand binding and transcriptional activation assays are not. Overall, ER transactivation assays demonstrate a high level of concordance (sensitivity and specificity) with the EDSP's ER binding assay. This is plausible and not unexpected since both assays are measuring the same functional endpoint, ER-ligand binding. Therefore, results from ER transactivation assays provide reliable and equivalent information to ER competitive radioligand binding assays.

- The ERα/β transactivation assay performed by Kojima *et al.* (2004) evaluated the same key biochemical mechanisms as the ERβ transactivation assay (OPPTS 890.1300). These steps include ER ligand binding, nuclear translocation, binding to the DNA responsive element, and reporter gene transactivation. Additionally, the sensitivity of the assay and dynamic range of Kojima *et al.* are comparable to the sensitivity and dynamic range of other ER reporter gene assays in the open literature and the OPPTS 890.1300 ER transactivation assay used to develop the OPPTS guideline.

- The *in vitro* ER transactivation assay performed by Petit *et al.* (1997) also provides data of the same nature and quality as OPPTS 890.1300 ER transactivation guideline. This assay assesses the key steps evaluated by OPPTS 890.1250 and OPPTS 890.1300 that include ER binding and the other key steps of DNA binding and gene transactivation.

Table 4. Evaluation of Data Submitted in Relation to the Estrogen Receptor Binding Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1250 - Estrogen Receptor Binding Assay	

- The *in vivo* assay reported by Xie *et al.* (2005) provides another confirmatory and sensitive mechanistic assessment of ER binding potential.

These *in vitro* and *in vivo* mechanistic findings that demonstrate no ER agonistic or antagonistic binding potential are further supported by a SAR analysis using methodology developed by EPA's Office of Research and Development. SAR analysis clearly shows no estrogenic potential for glyphosate and the comprehensive *in vivo* data available for glyphosate demonstrate no effects on any endpoint that is sensitive and specific to compounds that have estrogenic or antiestrogenic activity. A detailed review of the *in vivo* data used to evaluate estrogenicity is reviewed in the sections for the uterotrophic, pubertal assays and fish screening assays.

The data generated by Kojima *et al.* (2004) and Petit *et al.*. (1997) are functionally equivalent to both the Estrogen Competitive Receptor Binding (OPPTS 890.1250) and the ER Transcriptional Activation Assay (OPPTS 890.1300) defined in the EDSP Tier 1 test battery. Consequently, no additional *in vitro* screening should be required to evaluate the potential for ER agonism and antagonism"

The submission from **PETA** states that "In another study, glyphosate active was tested in two complementary assays:one measuring activation of the estrogen receptor from rainbow trout in a yeast system and the other evaluating vitellogenin production in a trout liver cell culture system. Glyphosate had *no estrogenic activity* in either assay (Petit *et al.*, (1997). The data indicate no effect *in vitro* on aromatase and estrogen *with perhaps a slight anti-androgenic effect for* GA. Adjuvants used in commercial formulations, however, may affect endocrine activity."

"In a female pubertal study, an increase in estrous cycle length from 4.9 to 5.4 days was reported in the high-dose female F344 rats (3393mg/kg/day or 50,000 ppm) (NTP 1992). F344 rats, however, are known to exhibit highly variable estrous cycle lengths (4 to 6 days) leading Morrissey *et al.*. (1988) to conclude that 'stages of the estrous cycle are so variable [in F344 rats] that they may not be useful in assessing potential toxicity.' Even if the estrous cycle length data were valid, they are of doubtful significance because the extremely high dosage associated with its occurrence. As no changes in sperm counts or estrous cycling were observed in mice treated at the same extremely high doses, it is concluded that glyphosate does not adversely affect sperm concentration or estrous cyclicity at any relevant dosage (NTP, 1992). It is also important to note that these dose levels are several orders of magnitude greater than any exposure ever likely to be experienced by humans."

Table 4. Evaluation of Data Submitted in Relation to the Estrogen Receptor Binding Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1250 - Estrogen Receptor Binding Assay

"After reviewing the existing data for both GA and commercially available formulations, there *is no evidence that the active ingredient causes endocrine disruption.* The mechanism of glyphosate activity is not related to endocrine pathways and should not modulate any endocrine activity and experimental results support this hypothesis. Immune response depression and sperm content reduction are endpoints which could potentially be connected with endocrine effects are also not shown to be caused *in vitro* and *in vivo* by glyphosate (Takacas 2002).

No effects were observed in numerous, multigeneration reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and its primary degradate AMPA are *not reproductive toxicants* and *do not perturb the endocrine system.*

The U.S. EPA (1998a) reviewed these studies and also concluded that there was *no evidence to suggest that glyphosate produces endocrine-modulating effects.* The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies.

The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies (Williams 2000).

Data is plentiful and well balanced, including many *in vitro* and *in vivo* studies, covering identical endpoints indicated in the EDSP. *No additional data is needed* to screen glyphosate active for endocrine disrupting activity because the data taken together clearly indicate no effects."

On review of the OSRI submitted, the Agency noted a number of deficiencies and therefore has outstanding questions about the potential for glyphosate to interact with the estrogen receptor.

Table 4. Evaluation of Data Submitted in Relation to the Estrogen Receptor Binding Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1250 - Estrogen Receptor Binding Assay	

- Gasnier *et al.* (2009) conducted a transcriptional activation study for anti-estrogenic activity with HepG2 cells. Glyphosate was negative for anti-estrogenic activity in this test system. In general, transcriptional activation assays and receptor binding assays are both needed to increase confidence in minimizing false negatives. Transcriptional activation assays do not measure receptor binding and involve post-binding events; therefore, they are not an adequate substitute for receptor binding assays.

- Kojima *et al.* (2004) in a transactivation assay, concluded that glyphosate, had neither estrogenic nor anti-estrogenic activity using transfected Chinese Hamster Ovary-K1 cells. In general, transcriptional activation assays and receptor binding assays are both needed to increase confidence in minimizing false negatives. Transcriptional activation assays do not measure receptor binding and involve post-binding events; therefore, they are not an adequate substitute for receptor binding assays

- Morrissey (1988) see Section IV of this report.

- The NTP (1992) subchronic toxicity study in rats did not measure binding of the chemical to the estrogen receptor.

- Petit *et al.* (1997) evaluated glyphosate in a reporter assay using recombinant yeast cells expressing trout estrogen receptor. Transcriptional activation assays do not measure receptor binding and involve post-binding events; therefore, this study is not an adequate substitute for Estrogen Receptor Binding Assay.

- Takacas *et al.*, (2002) see Section IV of this report.

- USEPA (1998a) see Section IV of this report.

- Walsh *et al* (2000) see Section IV of this report.

- Williams *et al.* (2000) see Section IV of this report.

- Xie (2005) studied the induction of rainbow trout vitellogenin. This study did not measure binding of the chemical to the estrogen receptor.

Table 4. Evaluation of Data Submitted in Relation to the Estrogen Receptor Binding Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1250 - Estrogen Receptor Binding Assay	
4. Conclusion: Based on the deficiencies and unanswered questions listed above, the data cited as OSRI did not satisfy the requirement for the Estrogen Receptor Binding Assay using Guideline 890.1250.	

[1] -- = not measured; X = indicates the endpoint was measured in the assay but does not indicate whether or not it satisfies the data requirement of the test order. See section 3 below for a detailed explanation; N/A = not applicable

Table 5. Evaluation of Data Submitted in Relation to the Estrogen Receptor Transcriptional Activation Assay

Chemical: Glyphosate	**PC Code: 417300**

890.1300 - Estrogen Receptor Transcriptional Activation Assay (Human Cell Line HeLa-9903)

1. EDSP Assay Endpoints[1]

Study Type / Literature Citation	**MRID No.**	**Bioluminescence measurements:**		
		EC50	**PC50**	**PC10**
Gasnier *et al.* (2009)	N/A	--	--	--
Kojima *et al.* (2004)	48033008	--	--	--
Petit *et al.* (1997)	N/A	--	--	--
NTP (1992) Subchronic – Rat	N/A	--	--	--
Takacas *et al.* (2002)	N/A	--	--	--
USEPA (1998a)	N/A	--	--	--
Williams *et al.* (2000)	N/A	--	--	--
Xie (2005)	N/A	--	--	--

2. Summary of Study Findings:

Study Type / Literature Citation	**MRID No.**	**Findings**
Gasnier *et al.* (2009)	N/A	See discussion below in Section 3.
Kojima *et al.* (2004)	48033008	See discussion below in Section 3.
NTP, (1992) Subchronic – Rat	N/A	See discussion below in Section 3.
Petit *et al.* (1997)	N/A	See discussion below in Section 3.
Takacas *et al.* (2002)	N/A	See Section IV of this report.
USEPA (1998a)	N/A	See Section IV of this report.
Williams *et al.* (2000)	N/A	See Section IV of this report.
Xie (2005)	N/A	See discussion below in Section 3.

3. Agency's Evaluation of the OSRI:

The submission provided by the Test Order Recipient states that "Data generated by Kojima *et al.*, (2004), Petit *et al.*, (1997) and Xie (2005) in peer-reviewed journals clearly and consistently demonstrate that glyphosate does not have an ER binding potential, either as an

Table 5. Evaluation of Data Submitted in Relation to the Estrogen Receptor Transcriptional Activation Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1300 - Estrogen Receptor Transcriptional Activation Assay (Human Cell Line HeLa-9903)

agonist or antagonist. The methodologies employed by Kojima and Petitin these *in vitro* test systems are comparable to the overall approach of the two Tier I ER binding assays of the EDSP- the ERα/β competitive radio ligand binding assay using rat uterine cytosol (OPPTS 890.1250) and the ERα transactivation assay (OPPTS 890.1300). Specifically, transcriptional activation assays performed by Kojima *et al.* (2004) are functionally equivalent to the ERα/β competitive radio ligand binding assay (OPPTS 890.1250) and the ERβ transactivation assay (OPPTS 890.1300) for the following reasons:

- Kojima *et al.*, (2004) evaluated the ability of glyphosate to agonize and antagonize ERα and ERβ binding, which is the purpose of the ERα/β competitive radio ligand binding assay (OPPTS 890.1250). The methodology of Kojima *et al.*, was capable of differentiating between ERα/β agonists and antagonists, which the ERα/β competitive radio ligand binding and transcriptional activation assays are not. Overall, ER transactivation assays demonstrate a high level of concordance (sensitivity and specificity) with the EDSP's ER binding assay. This is plausible and not unexpected since both assays are measuring the same functional endpoint, ER-ligand binding. Therefore, results from ER transactivation assays provide reliable and equivalent information to ER competitive radioligand binding assays.

- The ERα/β transactivation assay performed by Kojima *et al.*, (2004) evaluated the same key biochemical mechanisms as the ERβ transactivation assay (OPPTS 890.1300). These steps include ER ligand binding, nuclear translocation, binding to the DNA responsive element, and reporter gene transactivation. Additionally, the sensitivity of the assay and dynamic range of Kojima *et al.* are comparable to the sensitivity and dynamic range of other ER reporter gene assays in the open literature and the OPPTS 890.1300 ER transactivation assay used to develop the OPPTS guideline.

- The *in vitro* ER transactivation assay performed by Petit *et al.*, (1997) also provides data of the same nature and quality as OPPTS 890.1300 ER transactivation guideline. This assay assesses the key steps evaluated by OPPTS 890.1250 and OPPTS 890.1300 that include ER binding and the other key steps of DNA binding and gene transactivation.

- The *in vivo* assay reported by Xie *et al.* (2005) provides another confirmatory and sensitive mechanistic assessment of ER binding potential.

Table 5. Evaluation of Data Submitted in Relation to the Estrogen Receptor Transcriptional Activation Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1300 - Estrogen Receptor Transcriptional Activation Assay (Human Cell Line HeLa-9903)

These *in vitro* and *in vivo* mechanistic findings that demonstrate no ER agonistic or antagonistic binding potential are further supported by a SAR analysis using methodology developed by EPA's Office of Research and Development. SAR analysis clearly shows no estrogenic potential for glyphosate and the comprehensive *in vivo* data available for glyphosate demonstrate no effects on any endpoint that is sensitive and specific to compounds that have estrogenic or antiestrogenic activity. A detailed review of the *in vivo* data used to evaluate estrogenicity is reviewed in the sections for the uterotrophic, pubertal assays and fish screening assays.

The data generated by Kojima *et al.* (2004) and Petit *et al.* (1997) are functionally equivalent to both the Estrogen Competitive Receptor Binding (OPPTS 890.1250) and the ER Transcriptional Activation Assay (OPPTS 890.1300) defined in the EDSP Tier 1 test battery. Consequently, no additional *in vitro* screening should be required to evaluate the potential for ER agonism and antagonism."

The submission from **PETA** states that "In another study, glyphosate active was tested in two complementary assays: one measuring activation of the estrogen receptor from rainbow trout in a yeast system and the other evaluating vitellogenin production in a trout liver cell culture system. Glyphosate had *no estrogenic activity* in either assay (Petit *et al.*. (1997). The data indicate no effect *in vitro* on aromatase and estrogen *with perhaps a slight anti-androgenic effect for* GA. Adjuvants used in commercial formulations, however, may affect endocrine activity.

"In a female pubertal study, an increase in estrous cycle length from 4.9 to 5.4 days was reported in the high-dose female F344 rats (3393 mg/kg/day or 50,000 ppm) (NTP 1992). F344 rats, however, are known to exhibit highly variable estrous cycle lengths (4 to 6 days) leading Morrissey *et al.* (1988) to conclude that 'stages of the estrous cycle are so variable [in F344 rats] that they may not be useful in assessing potential toxicity.' Even if the estrous cycle length data were valid, they are of doubtful significance because the extremely high dosage associated with its occurrence. As no changes in sperm counts or estrous cycling were observed in mice treated at the same extremely high doses, it is concluded that glyphosate does not adversely affect sperm concentration or estrous cyclicity at any relevant dosage (NTP, 1992). It is also important to note that these dose levels are several orders of magnitude greater than any exposure ever likely to be experienced by humans."

After reviewing the existing data for both GA and commercially available formulations, there *is no evidence that the active ingredient causes endocrine disruption.* The mechanism of glyphosate activity is not related to endocrine pathways and should not modulate any endocrine activity and experimental results support this hypothesis. Immune response depression and sperm content reduction are endpoints which could potentially be connected with endocrine effects are also not shown to be caused *in vitro* and *in vivo* by glyphosate

Table 5. Evaluation of Data Submitted in Relation to the Estrogen Receptor Transcriptional Activation Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1300 - Estrogen Receptor Transcriptional Activation Assay (Human Cell Line HeLa-9903)	

(Takacas 2002).

No effects were observed in numerous, multigeneration reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and its primary degradate AMPA are *not reproductive toxicants* and *do not perturb the endocrine system.*

The U.S. EPA (1998a) reviewed these studies and also concluded that there was *no evidence to suggest that glyphosate produces endocrine-modulating effects*. The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies.

The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies (Williams 2000).

Data is plentiful and well balanced, including many *in vitro* and *in vivo* studies, covering identical endpoints indicated in the EDSP. *No additional data is needed* to screen glyphosate active for endocrine disrupting activity because the data taken together clearly indicate no effects."

On review of the OSRI submitted, the Agency noted a number of deficiencies and therefore has outstanding questions about the potential for glyphosate to transactivate the estrogen receptor:

- Gasnier *et al.* (2009) tested glyphosate for anti-estrogenic responses in an ER transcriptional activation assay in HepG2 cells. The authors reported that glyphosate was negative for anti-estrogenic activity in this test system. This study does not satisfy the test order because it did not evaluate estrogenic activity (only anti-estrogenic activity was investigated).

Table 5. Evaluation of Data Submitted in Relation to the Estrogen Receptor Transcriptional Activation Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1300 - Estrogen Receptor Transcriptional Activation Assay (Human Cell Line HeLa-9903)	
• Kojima *et al.* (2004) in a transactivation assay concluded that glyphosate tested at concentration range up to 10^{-5} M, had neither estrogenic nor anti-estrogenic activity using transfected Chinese Hamster Ovary-K1 cells. The most serious flaw of this study is that it does not contain enough detail to permit an independent review by EPA. Specifically no data were provided, and there was no information regarding cytotoxicity or solubility. In addition, the assay tested too narrow a range of concentrations. • The NTP (1992) subchronic toxicity study in rats did not measure activation of the estrogen receptor. • Petit *et al.* (1997) evaluated glyphosate in a reporter assay using recombinant yeast cells expressing trout estrogen receptor. Glyphosate was negative for activation of the ER at the concentrations tested (10^{-8} to 10^{-5} M). The assay tested too narrow a range of concentrations to be considered reliable. In addition, in general, yeast based assays are not an adequate substitute for the mammalian-based ERTA assays due to the yeast cell wall which some test substances may not be able to penetrate. This could result in false negatives. • Takacas *et al.*, (2002) see Section IV of this report. • USEPA (1998a) see Section IV of this report. • Williams *et al.* (2000) see Section IV of this report. • Xie (2005) studied the induction of rainbow trout vitellogenin. This study did not measure activation of the estrogen receptor.	
4. Conclusion: Based on the deficiencies and unanswered questions discussed above, the data cited as OSRI did not satisfy the requirement for the Estrogen Receptor Transcriptional Activation Assay using Guideline 890.1300.	

[1] -- = not measured; X = indicates the endpoint was measured in the assay but does not indicate whether or not it satisfies the data requirement of the test order. See section 3 below for a detailed explanation; N/A = not applicable

Table 6. Evaluation of Data Submitted in Relation to the Fish Short-Term Reproduction Assay

Chemical: Glyphosate	**PC Code: 417300**

890.1350 - Fish Short-Term Reproduction

1. EDSP Assay Endpoints[1]

Study Type / Literature Citation	**MRID No.**	**Reproductive Behavior and Secondary Sex Characteristics**						
		Fecundity	**Fertility**	**Vitellogenin**	**Sex Steroid Concentration**	**Secondary Sex Characteristics**	**Gonado-Somatic Index**	**Gonadal Histopathology**
Xie *et al.* (2005)	N/A	--	--	x	--	--	--	--
Avian Reproduction – Mallard Duck	00111953	x	x	--	--	--	--	--
Avian Reproduction – Bobwhite Quail	00108207	x	x	--	--	--	--	--
Bioconcentration Study in Bluegill Sunfish	41228302	--	--	--	--	--	--	--
Full Life Cycle Study in Fathead Minnows	00108171	x	x	--	--	--	--	--
Mann (1999)	N/A	--	--	--	--	--	--	--
Trotter (1990)	N/A	--	--	--	--	--	--	--
Takacs (2002)	N/A	--	--	--	--	--	--	--
McAllister and Forbis (1978)	00108205	--	--	--	--	--	--	--
EG & G Bionomics (1975) (acute toxicity portion)	00108171	--	--	--	--	--	--	--
Folmar, Sanders, and Julin (1979)	00162296	--	--	--	--	--	--	--

2. Summary of Study Findings:

Study Type / Literature Citation	**MRID No.**	**Findings**
Xie *et al.* (2005)	N/A	No observed vitellogenin induction in rainbow trout vitellogenin assay at 0.11 mg/L.

Table 6. Evaluation of Data Submitted in Relation to the Fish Short-Term Reproduction Assay		
Chemical: Glyphosate		**PC Code: 417300**
890.1350 - Fish Short-Term Reproduction		
Avian Reproduction – Mallard Duck	00111953	No treatment-related effects on endpoints related to reproduction, growth, or survival were seen at any dietary concentration tested (up to 1000 ppm).
Avian Reproduction – Bobwhite Quail	00108207	No treatment-related effects on endpoints related to reproduction, growth, or survival were seen at any dietary concentration tested (up to 1000 ppm).
Bioconcentration Study in Bluegill Sunfish	41228302	Glyphosate does not bioconcentrate in fish.
Full Life Cycle Study in Fathead Minnows	00108171	No treatment-related effects on endpoints related to reproduction, growth or survival were seen at any concentration level tested (up to 25.7 mg/L).
Mann (1999)	N/A	See Section IV of this report.
Trotter (1990)	N/A	See Section IV of this report.
Takacs (2002)	N/A	See Section IV of this report.
McAllister and Forbis (1978)	00108205	See Section IV of this report.
EG & G Bionomics (1975) (acute toxicity portion)	00108171	See Section IV of this report.
Folmar, Sanders, and Julin (1979)	00162296	See Section IV of this report.

3. Agency's Evaluation of the OSRI:

The submission provided by the Test Order Recipient states that "The methodology and information generated from a fish full life-cycle study provides a comprehensive evaluation of potential endocrine activity and provides information functionally equivalent to information obtained from the short-term fish reproduction assay. A summary of the relevant existing functionally equivalent data is summarized in the following table…"

"The fish full life-cycle study with glyphosate demonstrated no treatment-related effects on the survival, growth and egg production of first generation fish, or on hatchability, as well as survival and growth of second-generation eggs and fry, over an 8 month exposure period to concentrations as high 25.7 mg/L. Additionally, it has been shown in vivo that glyphosate does not induce production of vitellogenin nor induce an estrogen responsive reporter gene is a trout cell line, which is consistent with SAR analysis of glyphosate. The results from the fish full-life cycle study are also consistent with the results from subchronic and chronic tests with mammalian and avian species, which demonstrate no changes in endocrine function and all reproductive parameters. Based on these studies, sufficient information already exists to negate the need to perform the fish short-term reproduction

Table 6. Evaluation of Data Submitted in Relation to the Fish Short-Term Reproduction Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1350 - Fish Short-Term Reproduction

study. An additional fish reproduction screening with glyphosate is not necessary."

The submission from **PETA** states that "***Tables 2 and 3*** summarize available amphibian and fish data. GA is much less toxic than Roundup® to both fish and amphibians, and there is ***no evidence of endocrine activity***.

When comparing glyphosate active with a commercially available formula (e.g. Round-Up), researchers show that there is a significant difference in toxicity. When exposed to GA, *Oncorhynchus mykiss* (rainbow trout) had a 96-h LC50 range of 148-211 mg/L whereas the Roundup formulation was 14 mg/L (Takacs 2002)."

"Juvenile Rainbow trout (sex not specified) were exposed to 0.11 mg/L glyphosate active. Glyphosate did not induce elevated levels of vitellogenin in juvenile rainbow trout compared with control fish (Xie 2005)."

The Agency has reviewed the OSRI and has outstanding questions about the potential for glyphosate to interact with the HPG axis, given the following deficiencies:

- Xie *et al.* (2005) examined the effects of glyphosate on plasma vitellogenin in juvenile rainbow trout. The study used a 7-day exposure which is less than the 21-day exposure period for the Fish Short-Term Reproduction Assay, measured only one of the Assay parameters (i.e., vitellogenin), and tested only one concentration level.

- The bioconcentration studies in fish contain no endpoints related to the Fish Short-Term Reproduction Assay.

- The avian reproduction studies include measurements of fertility and fecundity but lack other endpoints related to reproduction, *e.g.*, vitellogenin concentrations, secondary sex characteristics, gonado-somatic index, and gonadal histopathology, which are measured in the Fish Short-Term Reproduction Assay.

- As with the avian studies, the fish full life cycle study includes measurements of fertility and fecundity but lack other endpoints related to reproduction that are measured in the Fish Short-Term Reproduction Assay.

Table 6. Evaluation of Data Submitted in Relation to the Fish Short-Term Reproduction Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1350 - Fish Short-Term Reproduction	
• Although certain elements of the endocrine system are conserved across vertebrate organisms, the absence of effects on apical endpoints of reproduction in birds and fish is not sufficient to preclude an interaction with the endocrine system. The avian reproduction studies also differ from the Fish Short-Term Reproduction Assay in the route and duration of exposure: fish are constantly exposed to the test chemical via uptake through the gills and integument, whereas birds are administered the chemical through the diet. Glyphosate consumed in the diet may be subject to different metabolic processes and therefore have different effects.	
4. Conclusion: Based on the deficiencies discussed above, the data cited as OSRI did not satisfy the requirement for the Fish Short-Term Reproduction Assay using Guideline 890.1350.	

[1] -- = not measured; X = indicates the endpoint was measured in the assay but does not indicate whether or not it satisfies the data requirement of the test order. See section 3 below for a detailed explanation; N/A = not applicable

Table 7. Evaluation of Data Submitted in Relation to the Hershberger Assay

Chemical: Glyphosate	**PC Code: 417300**

890.1400 - Hershberger Assay

1. EDSP Assay Endpoints[1]

Study Type / Literature Citation	**MRID No.**	**Tissue Weight**				
		Ventral Prostate	**Seminal Vesicle**	**LABC Muscle**	**Cowper's Glands**	**Glans Penis**
Morrissey *et al.* (1988)	N/A	--	--	--	--	--
Takacas *et al.* (2002)	N/A	--	--	--	--	--
USEPA (1998a)	N/A	--	--	--	--	--
Williams *et al.* (2000)	N/A	--	--	--	--	--
1981: Three-Generation Reproduction- Rat	00081674	--	--	--	--	--
1990: Two-Generation Reproduction – Rat	41621501	--	--	--	--	--
Developmental Toxicity – Rat	00046362	--	--	--	--	--
Developmental Toxicity – Rabbit	00046363	--	--	--	--	--
Chronic Toxicity/Carcinogenicity – Rat	41643801	--	--	--	--	--
Carcinogenicity – Mouse	00130406	--	--	--	--	--
Chronic Toxicity – Dog	00153374	--	--	--	--	--
Subchronic – Rat	40559401	--	--	--	--	--
Subchronic – Rat (NTP, 1992)	N/A	--	--	--	--	--
Subchronic – Mouse (NTP, 1992)	N/A	--	--	--	--	--
Subchronic – Mouse	00036803	--	--	--	--	--

2. Summary of Study Findings:

Study Type / Literature Citation	**MRID No.**	**Findings**
Morrissey *et al.* (1988)	N/A	See Section IV of this report.
Takacas *et al.* (2002)	N/A	See Section IV of this report.
USEPA (1998a)	N/A	See Section IV of this report.
Williams *et al.* (2000)	N/A	See Section IV of this report.
1981: Three-Generation Reproduction- Rat	00081674	No treatment-related effects were observed on male mating and fertility indices or fetal sex ratio over the three generations. No treatment-related

Table 7. Evaluation of Data Submitted in Relation to the Hershberger Assay		
Chemical: Glyphosate		**PC Code: 417300**
890.1400 - Hershberger Assay		
		changes in the absolute and relative testes weights or histopathological lesions of the testes and prostate glands were seen in the F0, F1, F2 adults or the F3b offspring.
1990: Two-Generation Reproduction – Rat	41621501	No treatment-related effects were observed in the male mating and fertility indices or fetal sex ratio. No treatment-related changes in absolute or relative testes weights or lesions of the testes, epididymides, seminal vesicle and prostate glands were seen in the F0 and F1 adults.
Developmental Toxicity – Rat	00046362	No treatment-related changes were seen in the number of corpora lutea, resorptions, fetal sex ratio, or soft tissue abnormalities at any dose. At 3500 mg/kg/day, there were significant ($p<0.05$) decreases in viable fetuses/dam (11.5 ± 4.12 vs. 14.4 ± 1.26) and total implantations/dam (12.8 ± 3.77 vs. 15.0 ± 1.67) when compared to controls.
Developmental Toxicity – Rabbit	00046363	No treatment-related changes were seen in the number of corpora lutea, implantation sites, early or late resorptions, number of live fetuses/litter, fetal sex ratio, or soft tissue abnormalities at any dose.
1981: Chronic Toxicity/Carcinogenicity – Rat	00093879	No treatment-related changes were seen in absolute or relative testes weights nor were there any treatment-related histopathological changes seen in the epididymides, seminal vesicles and prostate glands. An increased incidence of interstitial cell tumors of the testes were seen in at the high dose (6/50; 12%) when compared to controls (0/50) with the increase reaching statistical significance ($p=0.013$) being slightly higher than the highest concurrent control incidence (7%) and markedly higher than the overall historical control incidence of 4.5% (24/535 animals).
1990: Chronic Toxicity/Carcinogenicity – Rat	41643801	No treatment-related changes were seen in absolute or relative weights of the testes, epididymides and prostate glands. No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicle and the prostate glands.
Carcinogenicity – Mouse	00130406	No treatment-related changes were seen in absolute or relative weights of the testes. No treatment-related histopathological lesions were seen in the testes, epididymides and prostate glands.

Table 7. Evaluation of Data Submitted in Relation to the Hershberger Assay		
Chemical: Glyphosate		**PC Code: 417300**
890.1400 - Hershberger Assay		
Chronic Toxicity – Dog	00153374	No treatment-related changes were seen in absolute or relative weights of the testes nor were there any treatment-related histopathological lesions were seen in the testes.
Subchronic – Rat	40559401	No treatment-related changes were seen in absolute or relative weights of the testes and epididymides. No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicle and prostate glands.
Subchronic – Rat (NTP, 1992)		Sperm counts were significantly ($p < 0.01$) decreased (20%) at 1678 and 3393 mg/kg/day dose groups. Epididymal sperm motility, total spermatid head/testes, and total spermatid heads/g caudal tissue in the treated animals were not different from those of controls. No treatment-related changes were seen in left caudal, epididymal and testicular weights. Relative weights of the right testes were significantly increased at 1678 mg/kg/day dose (7%; $p < 0.05$) and at 3393 mg/kg/day dose (20%; $p < 0.01$) groups. Absolute weights in treated rats were comparable to the controls. No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicles, prostate and the preputial gland.
Subchronic – Mouse (NTP, 1992)		No treatment-related changes were seen in sperm concentration, motility, counts or morphology at any dose level. No treatment-related changes were seen in left caudal, epididymal and testicular weights. Relative weights of the right testes were significantly ($p < 0.01$) increased at 4776 mg/kg/day dose (10%) and at 10,780 mg/kg/day dose (18%) groups. Absolute weights in treated rats were comparable to the controls. No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicles, prostate and the preputial gland.
Subchronic – Mouse	00036803	No treatment-related changes were seen in absolute or relative weights of the testes. No treatment-related histopathological lesions were seen in the testes, epididymides and prostate glands.

Table 7. Evaluation of Data Submitted in Relation to the Hershberger Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1400 - Hershberger Assay

3. Agency's Evaluation of the OSRI:

The submission provided by the Test Order Recipient states that "Clear and consistent results from four multigenerational reproduction studies and two repeat dose toxicity studies on glyphosate provide strong scientific evidence that glyphosate is not an AR agonist, AR antagonist or a 5α-reductase inhibitor, and go beyond the five male sex gland weight endpoints assessed in the Hershberger assay. Based on this information, the Hershberger assay conducted according to OPPTS 890.1400 guideline is considered duplicative and unnecessary to characterize the potential for glyphosate to interact with androgen receptors or inhibit 5α-reductase *in vivo.*"

Summary of Findings Relative to the Hershberger Assay for Glyphosate

End Point in Hershberger Assay	Functionally Equivalent Information from E□isting Studies
Prostate/ventral weight	No differences in absolute or relative weights of the prostate or any treatment related effects regarding the prostate were noted in a subchronic rat study, a chronic rat study, four multigenerational rat studies.
Seminal Vesicles weights	No difference in absolute or relative weights or any gross or microscopic treatment related effects were noted in the seminal vesicles (where evaluated) in multigenerational studies.
LABC weights	LABC weight was not evaluated in any of the glyphosate studies. However, the absence of fertility issues and corroborative male reproductive organ weight data provides a substantial weight of evidence across multiple studies, suggesting no effect on LABC weights.
Bulbourethral (Cowper's) Gland	Bulbourethral gland weights were not measured in any of the glyphosate studies. However, the absence of fertility issues and corroborative male reproductive organ weight data provides a substantial weight of evidence across multiple studies, suggesting no effect on Bulbourethral weights. Glans penis weights were not measured in any of the glyphosate studies.
Glans Penis weight	Glans penis weights were not measured in any of the glyphosate studies. However, the absence of fertility issues and corroborative male reproductive organ weight data provides a substantial weight of evidence across multiple studies, suggesting no effect on glans penis weights.

Table 7. Evaluation of Data Submitted in Relation to the Hershberger Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1400 - Hershberger Assay

The submission from **PETA** states that "In a subchronic toxicity study conducted in rats by NTP (1992), reduced epididymal sperm concentrations(20% below control) were reported in F344 rats at both the 1638 mg/kg (25,000 ppm) and the 3393 mg/kg(50,000 ppm) levels. Nevertheless, all values were well within the normal range of sperm concentration values reported by the NTP in an analysis of their historical control data for these rodents (Morrissey *et al.*., 1988). As the apparent reductions were not related to dosage or accompanied by decreases in epididymal weights or testicular sperm numbers/weight, the relationship to treatment is doubtful. Moreover, *male fertility was not reduced* in the reproduction study even at the highest dietary level tested (30,000 ppm) (NTP 1992).

In a *pubertal* study, male weanling Wistar rats were dosed at 5, 50, or 250mg/kg of Roundup®. Bodyweight was not affected although a significant delay in puberty, significant weight decrease of adrenals, and a slight decrease in testosterone levels was seen in all three treatment groups. No pathological alterations of adrenals were seen and corticosterone and estradiol levels were not different researchers concluded that the direct action of the active ingredient and was not likely the major cause of the puberty delay and that glyphosate active did not present harmful effects on fertility, but instead showed effects for its adjuvant components. (Romano 2010). The data indicate that GA does not affect male fertility or hormone levels."

"After reviewing the existing data for both GA and commercially available formulations, there *is no evidence that the active ingredient causes endocrine disruption.* The mechanism of glyphosate activity is not related to endocrine pathways and should not modulate any endocrine activity and experimental results support this hypothesis. Immune response depression and sperm content reduction are endpoints which could potentially be connected with endocrine effects are also not shown to be caused *in vitro* and *in vivo* by glyphosate (Takacas 2002).

No effects were observed in numerous, multi-generation reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and its primary degradate AMPA are *not reproductive toxicants* and *do not perturb the endocrine system.*

The U.S. EPA (1998a) reviewed these studies and also concluded that there was *no evidence to suggest that glyphosate produces endocrine-modulating effects.* The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies.

Table 7. Evaluation of Data Submitted in Relation to the Hershberger Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1400 - Hershberger Assay	

The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies (Williams 2000).

Data is plentiful and well balanced, including many *in vitro* and *in vivo* studies, covering identical endpoints indicated in the EDSP. *No additional data is needed* to screen glyphosate active for endocrine disrupting activity because the data taken together clearly indicate no effects."

On review of the OSRI submitted, the Agency noted deficiencies and therefore has outstanding questions about the potential for glyphosate to disrupt the androgen pathway:

- The major difference between the cited studies and the Hershberger Assay is that the cited studies used intact animals. The Hershberger Assay uses the castrated male rat to increase the sensitivity of the overall test system to androgen-mediated effects.
- The Hershberger Assay and the cited OSRI differ in that the Hershberger Assay includes a measurement of the weight of the ventral prostate and four tissues that are male secondary sex organs (seminal vesicle, Cowper's gland, LABC muscle complex, and glans penis). The cited studies do not provide data on the weights of any of these specific androgen responsive tissues.
- In the NTP Subchronic Study with rats, decreased sperm counts and increased relative testes weights were seen at doses greater than the Limit Dose (i.e., 1678 and 3393 mg/kg/day).
- In the NTP Subchronic Study with mice, increased relative testes weights were seen at doses greater than the Limit Dose (i.e., 4776 and 10,780 mg/kg/day).
- In the Chronic Toxicity/Carcinogenicity Study (1981), interstitial cell tumors of the testes were seen at the high dose (31 mg/kg/day).

4. Conclusion: Based on the deficiencies and unanswered questions listed above, the data cited as OSRI did not satisfy the requirement for the Hershberger Assay using Guideline 890.1400.

[1] -- = not measured; X = indicates the endpoint was measured in the assay but does not indicate whether or not it satisfies the data requirement of the test order. See section 3 below for a detailed explanation; N/A = not applicable

Table 8. Evaluation of Data Submitted in Relation to the Female Pubertal Assay

Chemical: Glyphosate	**PC Code: 417300**

890.1450 - Female Pubertal Assay (Rat)

1. EDSP Assay Endpoints[1]		**Organ Weights**								
Study Type / Literature Citation	**MRID No.**	**Growth**	**Age and Weight at VO**	**Uterus**	**Ovaries**	**Thyroid**	**Liver**	**Kidneys**	**Pituitary**	**Adrenals**
Morrissey *et al.* (1988)	N/A	--	--	--	--	--	--	--	--	--
Takacas *et al.* (2002)	N/A	--	--	--	--	--	--	--	--	--
USEPA (1998a)	N/A	--	--	--	--	--	--	--	--	--
Williams *et al.* (2000)	N/A	--	--	--	--	--	--	--	--	--
1981: Three-Generation Reproduction- Rat	00081674	x	--	--	x	x	x	x	x	x
1990: Two-Generation Reproduction – Rat	41621501	x	--	--	--	--	x	x	X	x
Developmental Toxicity – Rat	00046362	x	--	--	--	--	--	--	--	--
Developmental Toxicity – Rabbit	00046363	x	--	--	--	--	--	--	--	--
1981: Chronic Toxicity/ Carcinogenicity – Rat	00093879	x	--	--	x	x	x	x	x	x
1990: Chronic Toxicity/ Carcinogenicity – Rat	41643801	x	--	--	--	--	x	x	--	--
Carcinogenicity – Mouse	00130406	x	--	--	x	--	x	x	--	x
Chronic Toxicity – Dog	00153374	x	--	--	x	x	x	x	x	x

Table 8. Evaluation of Data Submitted in Relation to the Female Pubertal Assay										
Chemical: Glyphosate					**PC Code: 417300**					
890.1450 - Female Pubertal Assay (Rat)										
Subchronic – Rat	40559401	x	--	--	--	--	x	x	--	--
Subchronic – Rat (NTP, 1992)	N/A	x	--	--	--	--	x	x	--	--
Subchronic – Mouse (NTP, 1992)	N/A	x	--	--	--	--	x	x	--	--
Subchronic – Mouse	00036803	x	--	--	x	--	x	x	--	--
Study Type/ Literature Citation	**MRID No.**	**Clinical Chemistry, Hormone, Pathology, Cyclicity [1]**								
		Histopathology				**Blood Chemistry**	**Hormones**		**Estrous Cyclicity (Age, Length & % of animals Cycling)**	
		Uterus	**Ovary**	**Thyroid**	**Kidney**		**T4**	**TSH**		
Morrissey *et al.* (1988)	N/A	--	--	--	--	--	--	--	--	
Takacas *et al.* (2002)	N/A	--	--	--	--	--	--	--	--	
USEPA (1998a)	N/A	--	--	--	--	--	--	--	--	
Williams *et al.* (2000)	N/A	--	--	--	--	--	--	--	--	
1981: Three-Generation Reproduction- Rat	00081674	x	x	x	x	--	--	--	--	
1990: Two-Generation Reproduction – Rat	41621501	x	x	--	x	--	--	--	--	
Developmental Toxicity – Rat	00046362	--	--	--	--	--	--	--	--	
Developmental Toxicity – Rabbit	00046363	--	--	--	--	--	--	--	--	

Table 8. Evaluation of Data Submitted in Relation to the Female Pubertal Assay									
Chemical: Glyphosate					**PC Code: 417300**				
890.1450 - Female Pubertal Assay (Rat)									
1981: Chronic Toxicity/ Carcinogenicity – Rat	00093879	x	x	x	x	x	--	--	--
1990: Chronic Toxicity/ Carcinogenicity – Rat	41643801	x	x	x	x	x	--	--	--
Carcinogenicity – Mouse	00130406	x	x	x	x	--	--	--	--
Chronic Toxicity – Dog	00153374	x	x	x	x	x	--	--	--
Subchronic – Rat	40559401	x	x	x	x	x	--	--	--
Subchronic – Rat (NTP, 1992)	N/A	x	x	x	x	--	--	--	--
Subchronic – Mouse (NTP, 1992)	N/A	x	x	x	x	--	--	--	--
Subchronic – Mouse	00036803	x	x	x	x	--	--	--	--
2. Summary of Study Findings:									
Study Type / Literature Citation	**MRID No.**	**Findings**							
Morrissey *et al.* (1988)	N/A	See Section IV of this report.							
Takacas *et al.* (2002)	N/A	See Section IV of this report.							
USEPA (1998a)	N/A	See Section IV of this report.							
Williams *et al.* (2000)	N/A	See Section IV of this report.							
1981: Three-Generation Reproduction- Rat	00081674	No treatment-related effects were observed on female mating and fertility indices, pregnancy or fetal sex ratio over the three generations. No treatment-related changes in absolute or relative weights of the ovaries, adrenal and pituitary glands of the parental (F0, F1 and F2) or the F3b offspring. No							

Table 8. Evaluation of Data Submitted in Relation to the Female Pubertal Assay		
Chemical: Glyphosate		**PC Code: 417300**
890.1450 - Female Pubertal Assay (Rat)		
		treatment-related histopathological lesions were seen in the ovaries, uterus, mammary, thyroid, pituitary and adrenal gland of the F0, F1 and F2 adults or the F3b offspring.
1990: Two-Generation Reproduction – Rat	41621501	No treatment-related effects were observed in female mating and fertility indices, gestation index, gestation length, live birth index, viability index, lactation index, parturition, or fetal sex ratio. No treatment-related changes in absolute or relative ovarian weights or histopathological lesions of the ovaries, uterus, vagina, mammary land were seen in the F0 and F1 adults.
Developmental Toxicity – Rat	00046362	No treatment-related changes were seen in the number of corpora lutea, resorptions, fetal sex ratio, or soft tissue abnormalities at any dose. At 3500 mg/kg/day, there were significant ($p<0.05$) decreases in viable fetuses/dam (11.5 ± 4.12 vs. 14.4 ± 1.26) and total implantations/dam (12.8 ± 3.77 vs. 15.0 ± 1.67) when compared to controls.
Developmental Toxicity – Rabbit	00046363	No treatment-related changes were seen in the number of corpora lutea, implantation sites, early or late resorptions, number of live fetuses/litter, fetal sex ratio, or soft tissue abnormalities at any dose.
1981: Chronic Toxicity/Carcinogenicity – Rat	00093879	No treatment-related changes were seen in absolute or relative weights of the ovaries, thyroid and adrenal glands. No treatment-related histopathological changes were seen in the ovaries, uterus, mammary glands, adrenal and pituitary glands. There was an increase in thyroid C-cell carcinomas in females only at the high dose (5/47; 11%) when compared to control females (1/47; 2%). No increases were seen in adenomas and the incidences of C-cell hyperplasia were comparable between the treated and the control groups.
1990: Chronic Toxicity/ Carcinogenicity – Rat	41643801	No treatment-related histopathological lesions were seen in the ovaries, uterus, mammary, adrenal and pituitary glands. The incidences of thyroid C-cell adenomas were increased in females at the mid (10%) and high (10%) dose groups compared to controls (3.3%). These increases were not considered to be treatment-related because: 1) none of the increases reached statistical significance; 2) there was no dose-response; 3) no increase in severity of grade or incidence in hyperplasia; 4) lack of progression to malignancy; and 4) the incidences were within the testing laboratories historical control ranges.
Carcinogenicity – Mouse	00130406	No treatment-related changes were seen in absolute or relative weights of the ovaries and adrenal glands. No treatment-related histopathological lesions were seen in the ovaries, uterus, mammary, thyroid, and adrenal and pituitary glands.

Table 8. Evaluation of Data Submitted in Relation to the Female Pubertal Assay		
Chemical: Glyphosate		**PC Code: 417300**
890.1450 - Female Pubertal Assay (Rat)		
Chronic Toxicity – Dog	00153374	No treatment-related changes were seen in absolute or relative weights of the ovaries, thyroid, adrenal and pituitary glands. No treatment-related histopathological lesions were seen in the ovaries, uterus, thyroid, adrenal and pituitary glands.
Subchronic – Rat	40559401	No treatment-related histopathological lesions were seen in the ovaries, uterus, cervix, mammary, thyroid, adrenal and pituitary glands.
Subchronic – Rat (NTP, 1992)	N/A	A significantly ($p<0.05$) longer estrous cycle length was seen in rats at the 3393 mg/kg/day (5.4 ± 0.21days) as compared to controls (4.9 ± 0.10 days). No treatment-related histopathological lesions were seen in the ovaries, uterus, vagina, clitoral glands, thyroid, adrenal and pituitary glands.
Subchronic – Mouse (NTP, 1992)	N/A	Not treatment-related changes were seen in estrous cycle length at any dose level. No treatment-related histopathological lesions were seen in the ovaries, uterus, vagina, clitoral glands, thyroid, adrenal and pituitary glands.
Subchronic – Mouse	00036803	No treatment-related changes were seen in absolute or relative ovarian weights nor were there any treatment-related histopathological lesions in the ovaries, uterus, mammary, thyroid, adrenal and pituitary glands.

3. Agency's Evaluation of the OSRI:

The submission provided by the Test Order Recipient states that "The lack of potential for glyphosate to interact with the female endocrine system has already been comprehensively addressed in four multigenerational rat reproduction studies conducted on glyphosate, with additional weight-of-evidence provided by subchronic and chronic toxicity studies on glyphosate. The EDSP Tier 1 assay, 'Pubertal Development and Thyroid Function in Intact Juvenile / Peripubertal Female Rats,' is capable of detecting estrogenic/anti-estrogenic activity of chemicals, or agents that alter pubertal development, via changes in steroidogenesis or hypothalamic-pituitary regulation of the ovary and thyroid homeostasis. The key endpoints evaluated in this study are also evaluated in the multigenerational reproduction, subchronic and/or chronic studies. The results from the multigenerational studies, which demonstrate no effects on pubertal development and thyroid function in the intact juvenile/peripubertal female rats, offer compelling evidence that glyphosate does not interact with the endocrine system. To require further testing on animals via the Female Pubertal assay to evaluate the endocrine interaction potential of glyphosate would provide no new evidence and be clearly redundant. *A summary of the findings from these studies as they relate to the endpoints evaluated in the Female Pubertal Assay is presented in the Table 5.*"

Table 8. Evaluation of Data Submitted in Relation to the Female Pubertal Assay

Chemical: Glyphosate	**PC Code: 417300**

890.1450 - Female Pubertal Assay (Rat)

Table 5. Summary of Findings Relative to the Female Pubertal Assay for Glyphosate

End Point Observations	**Observations**	**Microscopic Evaluation**
Age at VO	No change in age	N/A
Body Weight at VO	No change in body weight	N/A
Estrous Cyclicity	Slight marginal changes in number and mean cycle length at the high dose level (10,000 ppm); inconsistent across generations; considered not treatment related	
Ovaries	No change in absolute or relative weight	no treatment related effects
Thyroid	N/A	no treatment related effects
Kidney	No change in absolute or relative weight	no treatment related effects
Liver	No difference in absolute or relative weight in F0 and Fl b adults and F3b offspring. Slight decrease in relative weight in the F2b females attributed to slightly higher weight in the control group; non-dose related response	N/A
Adrenal	No difference in absolute or relative weight no treatment related effects in one study, and only slightly lower in mid-dose level of another; considered incidental	no treatment related effects
Pituitary	No change in absolute or relative weight	no treatment related effects
Clinical Chemistry	Small changes in some values; not considered to treatment related or biologically significant	

The submission by **PETA** states that "In a female pubertal study, an increase in estrous cycle length from 4.9 to 5.4 days was reported in the high-dose female F344 rats (3393mg/kg/day or 50,000 ppm) (NTP 1992). F344 rats, however, are known to exhibit highly variable estrous cycle lengths (4 to 6 days) leading Morrissey *et al.*, (1988) to conclude that 'stages of the estrous cycle are so variable [in F344 rats] that they may not be useful in assessing potential toxicity.' Even if the estrous cycle length data were valid, they are of doubtful significance because the extremely high dosage associated with its occurrence. As no changes in sperm counts or estrous cycling were observed in mice treated at the same extremely high doses, it is concluded that glyphosate does not adversely affect sperm concentration or estrous cyclicity at any relevant dosage (NTP, 1992). It is also important to note that these dose levels are several orders of magnitude greater than any exposure ever likely to be experienced by humans."

Table 8. Evaluation of Data Submitted in Relation to the Female Pubertal Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1450 - Female Pubertal Assay (Rat)

"After reviewing the existing data for both GA and commercially available formulations, there *is no evidence that the active ingredient causes endocrine disruption*. The mechanism of glyphosate activity is not related to endocrine pathways and should not modulate any endocrine activity and experimental results support this hypothesis. Immune response depression and sperm content reduction are endpoints which could potentially be connected with endocrine effects are also not shown to be caused *in vitro* and *in vivo* by glyphosate (Takacas 2002).

No effects were observed in numerous, multi-generation reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and its primary degradate AMPA are *not reproductive toxicants* and *do not perturb the endocrine system.*

The U.S. EPA (1998a) reviewed these studies and also concluded that there was *no evidence to suggest that glyphosate produces endocrine-modulating effects*. The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies.

The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies (Williams 2000).

Data is plentiful and well balanced, including many *in vitro* and *in vivo* studies, covering identical endpoints indicated in the EDSP. *No additional data is needed* to screen glyphosate active for endocrine disrupting activity because the data taken together clearly indicate no effects."

Table 8. Evaluation of Data Submitted in Relation to the Female Pubertal Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1450 - Female Pubertal Assay (Rat)

On review of the OSRI submitted, the Agency noted the following:

Although the OSRI cited for the estrogenic pathway mentions two 2-Generation Reproduction studies conducted in 2000 and 2007 [890.3800 Subdivision F Guideline requirements (i.e., Post-1998)], these studies have not been submitted to the Agency for review at the time this report was finalized. The glyphosate OSRI also cited a 3-Generation Reproduction Study conducted in 1981 and a 2-Generation Reproduction Study conducted in 1990 as well as a reproductive toxicity study in mice and rats that examined sperm parameters, vaginal cytology, organ weight and histopathology of the female reproductive organs. The following results were observed in the available studies:

- In the NTP reproductive toxicity study with rats, a longer estrous cycle length was seen only at a dose that is 3X the Limit Dose (3393 mg/kg/day). No effect on estrous cycle was seen in mice even at very high doses (>10,000 mg/kg/day).
- Vaginal opening which is a sensitive marker of sexual maturation and potential disruption of the estrogen pathway was not measured in the cited available 2-Generation Reproduction studies.
- No adverse effects on reproductive performance were seen in the cited available multi-generation reproduction studies.
- No treatment-related changes were seen in the absolute or relative ovarian weights across F0, F1b and F2b female adults and F3b weanlings in the 1981 Three-Generation in the F0 and F1a parents in the 1990 Two-Generation Reproduction studies.
- No treatment-related histopathological lesions were seen in the ovaries and uterus of the F0 parents, F1b parents, F2b parents and F3b offspring in the 1981 Three-Generation, in the F0 and F1a parents or the 1990 Two-Generation Reproduction studies.

On review of the OSRI submitted, the Agency noted the following for the HPT axis in females:

- Thyroid hormones measured in the Female Pubertal Tier 1 Assay were not available from the cited studies.
- Thyroid weights of the offspring were not measured in the cited available studies.

Table 8. Evaluation of Data Submitted in Relation to the Female Pubertal Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1450 - Female Pubertal Assay (Rat)

- In the 1981 chronic toxicity/carcinogenicity study in rats, thyroid C-cell carcinomas were non-significantly increased at the high dose (11%) compared to controls (2%). No increases in adenomas or corroborative non-neoplastic lesions were seen. These findings are not relevant to the HPT axis.

- Although thyroid histopathology was conducted and increased incidence of C-cell adenomas and/or carcinomas were observed in one study, it is not clear that thyroid follicular cell height and colloid area were evaluated histologically in any of the cited studies. The Tier 1 pubertal assays include specific instructions on the use of a 5-point grading scale for examination of follicular cell height and colloid area. These instructions include photomicrographs as reference. The 5-point grading scale provides for evaluation of subtle changes that may improve the sensitivity of the histopathological examination.

4. Conclusion:

Based on the deficiencies and unanswered questions listed above, the data cited as OSRI did not satisfy the requirement for the Female Pubertal Assay using Guideline 890.1450.

[1] -- = not measured; X = indicates the endpoint was measured in the assay but does not indicate whether or not it satisfies the data requirement of the test order. See section 3 below for a detailed explanation; N/A = not applicable

Table 9. Evaluation of Data Submitted in Relation to the Male Pubertal Assay

Chemical: Glyphosate	**PC Code: 417300**

890.1500 - Male Pubertal Assay (Rat)

1. EDSP Assay Endpoints[1]

Study Type / Literature Citation	MRID No.	Growth	Age and Weight at PPS	Organ Weights[2]										
				TE	EP	SV	VP	DLP	LABC	TH	LI	KDY	ADR	PIT
Morrissey *et al.* (1988)	N/A	--	--	--	--	--	--	--	--	--	--	--	--	--
Romanno *et al.* (2010)	N/A	--	--	--	--	--	--	--	--	--	--	--	--	--
Takacas *et al.* (2002)	N/A	--	--	--	--	--	--	--	--	--	--	--	--	--
USEPA (1998a)	N/A	--	--	--	--	--	--	--	--	--	--	--	--	--
Williams *et al.* (2000)	N/A	--	--	--	--	--	--	--	--	--	--	--	--	--
1981: Three-Generation Reproduction- Rat	00081674	x	--	x	--	--	--	--	--	--	x	x	x	x
1990: Two-Generation Reproduction – Rat	41621501	x	--	x	x	--	--	--	--	--	--	--	--	--
Developmental Toxicity – Rat	00046362	x	--	--	--	--	--	--	--	--	--	--	--	--
Developmental Toxicity – Rabbit	00046363	x	--	--	--	--	--	--	--	--	--	--	--	--
1981: Chronic Toxicity/Carcinogenicity – Rat	00093879	x	--	x	--	--	--	--	--	x	x	x	x	x
1990: Chronic Toxicity/ Carcinogenicity – Rat	41643801	x	--	x	x	--	--	--	--	--	x	x	--	--
Carcinogenicity – Mouse	00130406	x	--	x	--	--	--	--	--	--	x	x	x	--
Chronic Toxicity – Dog	00153374	x	--	x	--	--	--	--	--	x	x	x	x	x
Subchronic – Rat	40559401	x	--	x	--	--	--	--	--	--	x	x	--	--

Table 9. Evaluation of Data Submitted in Relation to the Male Pubertal Assay

Chemical: Glyphosate	**PC Code: 417300**

890.1500 - Male Pubertal Assay (Rat)

Subchronic – Rat (NTP, 1992)	N/A	x	--	x	--	--	--	--	--	--	x	x	--	--
Subchronic – Mouse (NTP, 1992)	N/A	x	--	x	--	--	--	--	--	--	x	x	--	--
Subchronic – Mouse	00036803	x	--	x	--	--	--	--	--	--	x	x	--	--

Study Type/ Literature Citation	**MRID No.**	**EDSP Assay Endpoints: Clinical Chemistry and Pathology[1]**							
		Blood Chemistry	**Hormones**			**Histopathology**			
			Testosterone	**T4**	**TSH**	**Epididymides**	**Testes**	**Thyroid**	**Kidney**
Morrissey *et al.* (1988)	N/A	--	--	--	--	--	--	--	--
Romanno *et al.* (2010)	N/A	--	--	--	--	--	--	--	--
Takacas *et al.* (2002)	N/A	--	--	--	--	--	--	--	--
USEPA (1998a)	N/A	--	--	--	--	--	--	--	--
Williams *et al.* (2000)	N/A	--	--	--	--	--	--	--	--
1981: Three-Generation Reproduction- Rat	00081674	--	--	--	--	x	x	x	x
1990: Two-Generation Reproduction – Rat	41621501	--	--	--	--	x	x	x	x
Developmental Toxicity – Rat	00046362	--	--	--	--	--	--	--	--
Developmental Toxicity – Rabbit	00046363	--	--	--	--	--	--	--	--
1981: Chronic Toxicity/ Carcinogenicity – Rat	00093879	x	--	--	--	x	x	x	x
1990: Chronic Toxicity/ Carcinogenicity – Rat	41643801	x	--	--	--	x	x	x	x
Carcinogenicity – Mouse	00130406	--	--	--	--	x	x	x	x

Table 9. Evaluation of Data Submitted in Relation to the Male Pubertal Assay									
Chemical: Glyphosate						**PC Code: 417300**			
890.1500 - Male Pubertal Assay (Rat)									
Chronic Toxicity – Dog	00153374	x	--	--	--	x	x	x	x
Subchronic – Rat	40559401	x	--	--	--	x	x	x	x
Subchronic – Rat (NTP)	N/A	--	--	--	--	x	x	x	x
Subchronic–Mouse (NTP)	N/A	--	--	--	--	x	x	x	x
Subchronic – Mouse	00036803	--	--	--	--	x	x	x	x

2. Summary of Study Findings:

Study Type / Literature Citation	MRID No.	Findings
Morrissey *et al.* (1988)	N/A	See Section IV of this report.
Romanno *et al.* (2010)	N/A	See Section IV of this report.
Takacas *et al.* (2002)	N/A	See Section IV of this report.
USEPA (1998a)	N/A	See Section IV of this report.
Williams *et al.* (2000)	N/A	See Section IV of this report.
1981: Three-Generation Reproduction- Rat	00081674	No treatment-related effects were observed on male mating and fertility indices or fetal sex ratio over the three generations. No treatment-related changes in absolute or relative weights of the testes, adrenal and pituitary glands of the parental (F0, F1 and F2) or the F3b offspring. No treatment-related histopathological lesions were seen in the testes, prostate, pituitary and adrenal gland of the F0, F1 and F2 adults or the F3b offspring.
1990: Two-Generation Reproduction – Rat	41621501	No treatment-related effects were observed in the male mating and fertility indices or fetal sex ratio. No treatment-related changes in absolute or relative testicular weights or histopathological lesions of the testes, epididymides, seminal vesicle and prostate glands in the F0 and F1 adults.
Developmental Toxicity – Rat	00046362	No treatment-related changes were seen in the number of corpora lutea, resorptions, fetal sex ratio, or soft tissue abnormalities at any dose. At 3500 mg/kg/day, there were significant ($p<0.05$) decreases in viable fetuses/dam (11.5 ± 4.12 vs. 14.4 ± 1.26) and total implantations/dam (12.8 ± 3.77 vs. 15.0 ± 1.67) when compared to controls.
Developmental Toxicity – Rabbit	00046363	No treatment-related changes were seen in the number of corpora lutea, implantation sites, early or late resorptions, number of live fetuses/litter, fetal sex ratio, or soft tissue abnormalities at any dose.

Table 9. Evaluation of Data Submitted in Relation to the Male Pubertal Assay		
Chemical: Glyphosate		**PC Code: 417300**
890.1500 - Male Pubertal Assay (Rat)		
1981:Chronic Toxicity/ Carcinogenicity – Rat	00093879	No treatment-related changes were seen in absolute or relative weights of the testes, thyroid and adrenal glands. No treatment-related histopathological changes were seen in the epididymides, seminal vesicles, prostate, adrenal and pituitary glands. • There was an increase in the incidences of interstitial cell tumors of the testes at the high dose (6/50; 12%) when compared to controls (0/50) that reached statistical significance (p=0.013) and was slightly higher than the highest concurrent control incidence (7%) and markedly higher than the overall historical control incidence of 4.5% (24/535 animals).
1990:Chronic Toxicity/ Carcinogenicity – Rat	41643801	No treatment-related changes were seen in absolute or relative weights of the testes, epididymides and prostate glands. No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicle, prostate, adrenal and pituitary glands. • The incidences of thyroid C-cell adenomas were increased in males at the mid (13.8%) and high dose (11.7%) groups and in females at the mid (10%) and high (10%) dose groups compared to control males (3.3%) and females (3.3%). These increases were not considered to be treatment-related because: 1) none of the increases reached statistical significance; 2) absence of dose-response; 3) no increase in severity of grade or incidence in hyperplasia; 4) lack of progression to malignancy; and 4) the incidences were within the testing laboratories historical control ranges.
Carcinogenicity – Mouse	00130406	No treatment-related changes were seen in absolute or relative weights of the testes and adrenal glands. No treatment-related histopathological lesions were seen in the testes, epididymides, prostate, thyroid, adrenal and pituitary glands.
Chronic Toxicity – Dog	00153374	No treatment-related changes were seen in absolute or relative weights of the testes, thyroid, adrenal and pituitary glands. No treatment-related histopathological lesions were seen in the testes, thyroid, adrenal and pituitary glands.
Subchronic – Rat	40559401	No treatment-related changes were seen in absolute or relative weights of the testes and epididymides. No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicle, prostate, ovaries, uterus, cervix, mammary, thyroid, adrenal and pituitary glands.
Subchronic – Rat (NTP, 1992)	N/A	Sperm counts were significantly ($p < 0.01$) decreased (20%) at 1678 and 3393 mg/kg/day dose groups. Epididymal sperm motility, total spermatid head/testes, and total spermatid heads/g caudal tissue in the treated animals were not different from those of controls. No treatment-related changes were seen in left caudal, epididymal and testicular weights. Relative weights of the right testes were significantly increased

Table 9. Evaluation of Data Submitted in Relation to the Male Pubertal Assay		
Chemical: Glyphosate		**PC Code: 417300**
890.1500 - Male Pubertal Assay (Rat)		
		at 1678 mg/kg/day dose (7%; p <0.05) and at 3393 mg/kg/day dose (20%; p <0.01) groups. Absolute weights in treated rats were comparable to the controls. No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicles, prostate, preputial gland, thyroid, adrenal and pituitary glands.
Subchronic – Mouse (NTP, 1992)	N/A	No treatment-related changes were seen in sperm concentration, motility, counts or morphology at any dose level. No treatment-related changes were seen in left caudal, epididymal and testicular weights. Relative weights of the right testes were significantly (p <0.01) increased at 4776 mg/kg/day dose (10%) and at 10,780 mg/kg/day dose (18%) groups. Absolute weights in treated rats were comparable to the controls. No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicles, prostate, preputial, thyroid, adrenal and pituitary glands.
Subchronic – Mouse	00036803	No treatment-related changes were seen in absolute or relative weights of the testes nor were there any treatment-related histopathological lesions in the testes, epididymides, prostate, thyroid, adrenal and pituitary glands.

3. Agency's Evaluation of the OSRI:

The submission provided by the Test Order Recipient states that "The lack of potential for glyphosate to interact with the male endocrine system has already been comprehensively addressed in four multi generational rat reproduction studies conducted on glyphosate, with additional weight-of-evidence provided by subchronic and chronic studies on glyphosate. The EDSP Tier 1 assay, 'Pubertal Development and Thyroid Function in Intact Juvenile / Peripubertal Male Rats,' is capable of detecting anti thyroid, androgenic, or antiandrogenic (androgen receptor or steroid-enzyme-mediated) activity of chemicals, or agents that alter pubertal development via changes in gonadotropins, prolactin, or hypothalamic function. All of the endpoints evaluated in this EDSP assay are also evaluated in the multigenerational reproduction, subchronic and/or chronic studies, or can be evaluated based on other information from these studies. The results from the multigenerational studies, which demonstrate no effects on pubertal development and thyroid function in the intact juvenile/peripubertal male rats, offer compelling evidence that glyphosate does not interact with the endocrine system. To require further testing on animals via the Male Pubertal assay to evaluate the endocrine interaction potential of glyphosate would provide no new evidence and clearly be redundant *A summary of the findings from these studies as they relate to the endpoints evaluated in the Male Pubertal assay is presented in Table 7."*

Table 9. Evaluation of Data Submitted in Relation to the Male Pubertal Assay

Chemical: Glyphosate	**PC Code: 417300**

890.1500 - Male Pubertal Assay (Rat)

Table 7. Summary of Findings Relative to the Male Pubertal Assay for Glyphosate

End Point	Observations	Microscopic Evaluation
Growth, Body weight	Reduction in body weight limited to high dose group as a result of reduced food consumption due to reduced palatability, and not due to endocrine effects	
Age at preputial separation	No glyphosate related change in age at preputial separation doses at or below the limit dose of 1,000 mg/kg/day	N/A
Body weight at preputial separation	Mean body weight decrease at 10,000 ppm and increase at 15,000 ppm. Both doses exceeded the limit dose of 1,000 mg/kg/day during sexual maturation; however, no other related endocrine effects were noted in sex organ weight differences, therefore the weight of the evidence is that body weight changes at sexual maturation were not related to endocrine effects	N/A
Testes weight	No change in absolute or relative weight in one rat reproduction study, and only a slight increase in relative weight (not absolute weight) of the 30,000 ppm test group (high dose) due to a lower mean terminal body weight	No treatment related effects
Epididymides Weight	No change in absolute or relative weight	No treatment related effects
Prostate Weight	No change in absolute or relative weight	No treatment related effects
Seminal Vesicles weight	No change in absolute or relative weight	No treatment related effects
Testosterone	No other related endocrine effects observed in the multigenerational reproduction studies	N/A
Thyroid Weight	N/A	No treatment related effects
TSH	No other related endocrine effects observed in the multigenerational reproduction studies	N/A
Thyroxine (T4)	No other related endocrine effects observed in the multigenerational reproduction studies	N/A
Kidney Weight	Increase in relative weight only in the FO male at the high dose; no change in the Fl male or in any adult males or offspring in the three generation reproduction study	N/A
Liver Weight	Inconsistent findings (decrease in absolute and increase in relative weights) in one reproduction study were considered incidental and unrelated to endocrine effects; no changes in the three generation reproduction study in males	N/A
Adrenal Weight	No change in absolute or relative weight	No treatment related effects
Pituitary Weight	No change in absolute or relative weight	
Clinical Chemistry	Small changes in some values; not considered to be treatment related or biologically significant	No treatment related effects

Table 9. Evaluation of Data Submitted in Relation to the Male Pubertal Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1500 - Male Pubertal Assay (Rat)

The submission from **PETA** states that "In a subchronic toxicity study conducted in rats by NTP (1992), reduced epididymal sperm concentrations (20% below control) were reported in F344 rats at both the 1638 mg/kg (25,000 ppm) and the 3393 mg/kg (50,000 ppm) levels. Nevertheless, all values were well within the normal range of sperm concentration values reported by the NTP in an analysis of their historical control data for these rodents (Morrissey *et al..*, 1988). As the apparent reductions were not related to dosage or accompanied by decreases in epididymal weights or testicular sperm numbers/weight, the relationship to treatment is doubtful. Moreover, *male fertility was not reduced* in the reproduction study even at the highest dietary level tested (30,000 ppm) (NTP 1992). In a *pubertal* study, male weanling Wistar rats were dosed at 5, 50, or 250 mg/kg of Roundup®. Bodyweight was not affected although a significant delay in puberty, significant weight decrease of adrenals, and a slight decrease in testosterone levels was seen in all three treatment groups. No pathological altercations of adrenals were seen and corticosterone and estradiol levels were not different researchers concluded that the direct action of the active ingredient and was not likely the major cause of the puberty delay and that glyphosate active did not present harmful effects on fertility, but instead showed effects for its adjuvant components. (Romano 2010). The data indicate that GA does not affect male fertility or hormone levels.

After reviewing the existing data for both GA and commercially available formulations, there *is no evidence that the active ingredient causes endocrine disruption.* The mechanism of glyphosate activity is not related to endocrine pathways and should not modulate any endocrine activity and experimental results support this hypothesis. Immune response depression and sperm content reduction are endpoints which could potentially be connected with endocrine effects are also not shown to be caused *in vitro* and *in vivo* by glyphosate (Takacas 2002).

No effects were observed in numerous, multi-generation reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and its primary degradate AMPA are *not reproductive toxicants* and *do not perturb the endocrine system.*

The U.S. EPA (1998a) reviewed these studies and also concluded that there was *no evidence to suggest that glyphosate produces endocrine-modulating effects*. The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies.

The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies (Williams 2000).

Table 9. Evaluation of Data Submitted in Relation to the Male Pubertal Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1500 - Male Pubertal Assay (Rat)

Data is plentiful and well balanced, including many *in vitro* and *in vivo* studies, covering identical endpoints indicated in the EDSP. *No additional data is needed* to screen glyphosate active for endocrine disrupting activity because the data taken together clearly indicate no effects."

On review of the OSRI submitted, the Agency noted the following:

Although the OSRI cited for the androgen pathway mentions two 2-Generation Reproduction studies conducted in 2000 and 2007 [890.3800 Subdivision F Guideline requirements (i.e., Post-1998)], these studies have not been submitted to the Agency for review at the time this report was finalized. In addition, the glyphosate OSRI also cited a 3-Generation Reproduction study conducted in 1981 and a 2-Generation Reproduction study conducted in 1990 as well as a reproductive toxicity study in mice and rats that examined sperm parameters and organ weight and histopathology of the male reproductive organs. The following results were observed:

- None of the cited available studies measured age and weight of the offspring at the time of PPS. This is a sensitive and critical measure of sexual maturation.
- In the NTP reproductive toxicity study with rats, sperm counts were decreased in rats only at doses greater than the Limit Dose (1678 and 3393 mg/kg/day). Epididymal sperm motility, total spermatid head/testes, and total spermatid heads/g caudal tissue in the treated animals were not different from those of controls.
- In the reproductive toxicity study with mice, no treatment-related changes were seen in sperm concentration, motility, counts or morphology at any dose level.
- No changes in absolute or relative testicular weights were seen in the F0, F1b and F2b adult males and F3b weanlings in the 1981 Three-Generation or in the F0 and F1a males in the 1990 Two-Generation Reproduction studies.
- No changes in the absolute or relative weights of the epididymides or the prostate glands were seen in the cited available studies.
- No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicles or the prostate glands F0, F1b and F2b adult males and F3b weanlings in the 1981 Three-Generation or in the F0 and F1a males in the 1990 Two-Generation Reproduction studies.

Table 9. Evaluation of Data Submitted in Relation to the Male Pubertal Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1500 - Male Pubertal Assay (Rat)

- While some of the accessory sex organs weighed in the Male Pubertal Assay have been weighed, other such organs were not measured (e.g., levator ani/bulbocavernosus muscle complex) or were not measured appropriately in the cited Part 158 studies (ventral prostate and dorsolateral prostate weighed separately). Having measures of these androgen sensitive tissues at the critical life stage in peri-pubertal animals increases the possibility of identifying subtle or weak endocrine disruptors.

- In the 1981 Chronic Toxicity/Carcinogenicity study in rats, interstitial cell tumors of the testes were increased (p=0.013) at the high dose (12%) when compared to controls (0%).

- Testosterone, which is measured in the Tier 1 Male Pubertal Assay, was not measured in the cited Part 158 studies

On review of the OSRI submitted, the Agency noted the following regarding potential for glyphosate to disrupt the HPT axis in males:

- Thyroid hormones measured in the Male Pubertal Tier 1 Assay provide information which is not available from the cited studies.

- Thyroid weights in offspring were not measured in the cited available studies.

- In the 1990 Chronic Toxicity/Carcinogenicity study in rats, there was a non-significantly increase in thyroid C-cell adenomas at the mid (13.8%) and high (11.7%) dose groups when compared to controls (3.3%). No carcinomas or non-neoplastic lesions were seen. These findings are not relevant to the HPT axis.

- Although thyroid histopathology was conducted and increased incidence of C-cell adenomas and/or carcinomas were observed in one study, it is not clear that thyroid follicular cell height and colloid area were evaluated histologically in any of the cited studies. The Tier 1 pubertal assays include specific instructions on the use of a 5-point grading scale for examination of follicular cell height and colloid area. These instructions include photomicrographs as reference. The 5-point grading scale provides for evaluation of subtle changes that may improve the sensitivity of the histopathological examination.

Table 9. Evaluation of Data Submitted in Relation to the Male Pubertal Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1500 - Male Pubertal Assay (Rat)	
4. Conclusion: Based on the deficiencies and unanswered questions listed above, the data cited as OSRI did not satisfy the requirement for the Male Pubertal Assay using Guideline 890.1500.	

[1] -- = not measured; X = indicates the endpoint was measured in the assay but does not indicate whether or not it satisfies the data requirement of the test order. See section 3 below for a detailed explanation; N/A = not applicable

[2]TE = Testes, EP = Epididymides; SV = Seminal Vesicle; VP= Ventral Prostate; DLP= Dorsolateral Prostate; LABC= levator ani-bulbocavernosus muscle complex; TH= Thyroid; LI= Liver; KDY = Kidney; ADR= Adrenal; PIT= Pituitary

Table 10. Evaluation of Data Submitted in Relation to the Steroidogenesis Assay				
Chemical: Glyphosate		**PC Code: 417300**		
890.1550 - Steroidogenesis Assay (Human Cell Line – H295R)				
1. EDSP Assay Endpoints[1]				
Study Type / Literature Citation	**MRID No.**	**17β- Estradiol Content**	**Testosterone Content**	**Cell Viability**
Morrissey *et al.* (1988)	N/A	--	--	--
NTP (1992)	N/A	--	--	--
Richard *et al.* (2005)	N/A	--	--	--
Romano *et al.* (2010)	N/A	--	--	--
Takacas *et al.* (2002)	N/A	--	--	--
USEPA (1998a)	N/A	--	--	--
Walsh *et al.* (2000)	N/A	--	--	--
Williams *et al.* (2000)	N/A	--	--	--
2. Study Type / Literature Citation	**MRID No.**	**Findings**		
Morrissey *et al.* (1988)	N/A	See Section IV of this report.		
NTP (1992)	N/A	See discussion below in Section 3.		
Richard *et al.* (2005)	N/A	See discussion below in Section 3.		
Romano *et al.* (2010)	N/A	See Section IV of this report.		
Takacas *et al.* (2002)	N/A	See Section IV of this report.		
USEPA (1998a)	N/A	See Section IV of this report.		
Walsh *et al.* (2000)	N/A	See Section IV of this report.		
Williams *et al.* (2000)	N/A	See Section IV of this report.		
3. Agency's Evaluation of the OSRI: The submission provided by the Test Order Recipient states that "*In vitro* data assessing potential impacts on the complete steriodogenic pathway are not available for glyphosate; however, data that is functionally equivalent to the *in vitro* steroidogenesis assay is provided by endpoints included in rat multigenerational studies. Specifically, it was confirmed in the most recent multigenerational studies that there were no treatment related effects at high dietary exposure levels on endpoints that would be impacted by an alteration of steroidogenesis which include:				

Table 10. Evaluation of Data Submitted in Relation to the Steroidogenesis Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1550 - Steroidogenesis Assay (Human Cell Line – H295R)

- testicular/uterine/ovary weights and histopathology
- size, function and histopathology of sex organs
- fertility
- anogenital distance
- sperm count and mobility
- ovarian evaluation for follicles and corpa lutea
- anogenital distance
- age and weight at preputial separation and vaginal opening
- number of implantation sites and lactation.

Glyphosate-related reductions in body weight were recorded only in F1 males at the HDT, but there is no evidence that this reduction is an endocrine-mediated effect. Additionally, there was no effect on several endpoints that would be impacted by inhibition of steroidogenesis following treatment at up to the HDT. The information from these *in vivo* studies with glyphosate provides compelling evidence that glyphosate has no effect on steroidogenesis."

The submission from **PETA** states that "In an *in vitro* assay, researchers used mouse MA-10 Leydig tumor cells to study the molecular events involved in pesticide-induced alterations in steroid hormone biosynthesis. Roundup significantly ($p < 0.001$) disrupted steroidogenesis over time without inducing a parallel decrease in total protein synthesis. Interestingly, the active ingredient in Roundup®, GA, *did not alter steroidogenesis* or total protein synthesis at any dose tested (0-100 μg/mL). Researchers indicated that Roundup® decreased steroidogenesis by disrupting StAR expression post-transcriptionally (Walsh 2000)."

"In a subchronic toxicity study conducted in rats by NTP (1992), reduced epididymal sperm concentrations (20% below control) were reported in F344 rats at both the 1638 mg/kg (25,000 ppm) and the 3393 mg/kg (50,000 ppm) levels. Nevertheless, all values were well within the normal range of sperm concentration values reported by the NTP in an analysis of their historical control data for these rodents (Morrissey *et al.*, 1988). As the apparent reductions were not related to dosage or accompanied by decreases in epididymal weights or testicular sperm numbers/weight, the relationship to treatment is doubtful. Moreover, ***male fertility was not reduced*** in the reproduction study even at the highest dietary level tested (30,000 ppm) (NTP 1992).

Table 10. Evaluation of Data Submitted in Relation to the Steroidogenesis Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1550 - Steroidogenesis Assay (Human Cell Line – H295R)

In a ***pubertal*** study, male weanling Wistar rats were dosed at 5, 50, or 250mg/kg of Roundup®. Body weight was not affected although a significant delay in puberty, significant weight decrease of adrenals, and a slight decrease in testosterone levels was seen in all three treatment groups. No pathological altercations of adrenals were seen and corticosterone and estradiol levels were not different. Researchers concluded that the direct action of the active ingredient and was not likely the major cause of the puberty delay and that glyphosate active did not present harmful effects on fertility, but instead showed effects for its adjuvant components. (Romano 2010).

The data indicate that GA does not affect male fertility or hormone levels."

"After reviewing the existing data for both GA and commercially available formulations, there *is no evidence that the active ingredient causes endocrine disruption.* The mechanism of glyphosate activity is not related to endocrine pathways and should not modulate any endocrine activity and experimental results support this hypothesis. Immune response depression and sperm content reduction are endpoints which could potentially be connected with endocrine effects are also not shown to be caused *in vitro* and *in vivo* by glyphosate (Takacas 2002).

No effects were observed in numerous, multigeneration reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and its primary degradate AMPA are ***not*** *reproductive toxicants* and *do not perturb the endocrine system.*

The U.S. EPA (1998a) reviewed these studies and also concluded that there was *no evidence to suggest that glyphosate produces endocrine-modulating effects*. The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies. The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies (Williams 2000).

Data is plentiful and well balanced, including many *in vitro* and *in vivo* studies, covering identical endpoints indicated in the EDSP. *No additional data is needed* to screen glyphosate active for endocrine disrupting activity because the data taken together clearly indicate no effects."

Table 10. Evaluation of Data Submitted in Relation to the Steroidogenesis Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1550 - Steroidogenesis Assay (Human Cell Line – H295R)

On review of the OSRI submitted, the Agency noted deficiencies and has remaining questions about the potential for glyphosate to disrupt steroidogenesis.

- Gasnier *et al.* (2009) evaluated glyphosate for ER and AR transcriptional activation and effects on aromatase activity, but did not evaluate the chemical for steroid synthesis.
- Kojima *et al.* (2004) tested glyphosate in transcriptional activation assays for AR and ER using CHO-K1 cells. The study did not evaluate steroid synthesis.
- Morrissey (1988) see Section IV of this report.
- The NTP (1992) subchronic toxicity study in rats did not evaluate steroid synthesis.
- Petit *et al.* (1997) studied trout ER transactivation in a yeast assay and hepatic cell assays, but did not evaluate steroid synthesis.
- Richard *et al.* (2005) tested glyphosate and several product formulations in JEG-1 cells using radioimmunoassay (RIA) as the method of detecting reaction products. Induction of mRNA was also followed and aromatase activity was also assessed using the placental microsomal assay by measuring the formation of tritiated water. The study did not evaluate steroid synthesis.
- Walsh *et al.* (2000) See Section IV of this report.
- Williams *et al.* (2000) see Section IV of this report.
- None of the cited studies measure key enzymes responsible for steroid synthesis or alterations in estradiol and testosterone concentrations, which is the information that would be obtained by the Tier 1 Steroidogenesis Assay.

Table 10. Evaluation of Data Submitted in Relation to the Steroidogenesis Assay	
Chemical: Glyphosate	**PC Code: 417300**
890.1550 - Steroidogenesis Assay (Human Cell Line – H295R)	
4. Conclusion: Based on the deficiencies discussed above, the data cited as OSRI did not satisfy the requirement for the Steroidogenesis Assay using Guideline 890.1550.	

[1] -- = not measured; X = indicates the endpoint was measured in the assay but does not indicate whether or not it satisfies the data requirement of the test order. See section 3 below for a detailed explanation; N/A = not applicable

Table 11. Evaluation of Data Submitted in Relation to the Uterotrophic Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1600 - Uterotrophic Assay (Rat)

1. EDSP Assay Endpoints[1]

Study Type / Literature Citation	MRID No.	Uterus Weight
Morrissey *et al.* (1988)	N/A	--
Takacas *et al.* (2002)	N/A	--
USEPA (1998a)	N/A	--
Williams *et al.* (2000)	N/A	--
1981: Three-Generation Reproduction- Rat	00081674	--
1990: Two-Generation Reproduction – Rat	41621501	--
Developmental Toxicity – Rat	00046362	--
Developmental Toxicity – Rabbit	00046363	--
1981: Chronic Toxicity/Carcinogenicity – Rat	00093879	--
1990: Chronic Toxicity/ Carcinogenicity – Rat	41643801	--
Carcinogenicity – Mouse	00130406	--
Chronic Toxicity – Dog	00153374	--
Subchronic – Rat	40559401	--
Subchronic – Rat (NTP, 1992)	N/A	--
Subchronic – Mouse (NTP, 1992)	N/A	--
Subchronic – Mouse	00036803	--

2. Summary of Study Findings:

Study Type / Literature Citation	MRID No.	Findings
Morrissey *et al.* (1988)	N/A	See Section IV of this report.
Takacas *et al.* (2002)	N/A	See Section IV of this report.
USEPA (1998a)	N/A	See Section IV of this report.
Williams *et al.* (2000)	N/A	See Section IV of this report.
1981: Three-Generation Reproduction- Rat	00081674	No treatment-related effects were observed on female mating and fertility indices, pregnancy or fetal sex ratio over the three generations. No

Table 11. Evaluation of Data Submitted in Relation to the Uterotrophic Assay		
Chemical: Glyphosate		**PC Code: 417300**
890.1600 - Uterotrophic Assay (Rat)		
		treatment-related changes in absolute or relative weights of the ovaries, adrenal and pituitary glands of the parental (F0, F1 and F2) or the F3b offspring. No treatment-related histopathological lesions were seen in the ovaries, uterus, mammary, thyroid, pituitary and adrenal gland of the F0, F1 and F2 adults or the F3b offspring.
1990: Two-Generation Reproduction – Rat	41621501	No treatment-related effects were observed in female mating and fertility indices, gestation index, gestation length, live birth index, viability index, lactation index, parturition, or fetal sex ratio. No treatment-related changes in absolute or relative ovarian weights or histopathological lesions of the ovaries, uterus, vagina, mammary gland were seen in the F0 and F1 adults.
Developmental Toxicity – Rat	00046362	No treatment-related changes were seen in the number of corpora lutea, resorptions, fetal sex ratio, or soft tissue abnormalities at any dose. At 3500 mg/kg/day, there were significant ($p<0.05$) decreases in viable fetuses/dam (11.5 ± 4.12 vs. 14.4 ± 1.26) and total implantations/dam (12.8 ± 3.77 vs. 15.0 ± 1.67) when compared to controls.
Developmental Toxicity – Rabbit	00046363	No treatment-related changes were seen in the number of corpora lutea, implantation sites, early or late resorptions, number of live fetuses/litter, fetal sex ratio, or soft tissue abnormalities at any dose.
1981: Chronic Toxicity/Carcinogenicity – Rat	00093879	No treatment-related changes were seen in absolute or relative weights of the ovaries, thyroid and adrenal glands. No treatment-related histopathological changes were seen in the ovaries, uterus, mammary glands, adrenal and pituitary glands. There was an increase in thyroid C-cell carcinomas in females only at the high dose (5/47; 11%) when compared to control females (1/47; 2%). No increases were seen in adenomas and the incidences of C-cell hyperplasia were comparable between the treated and the control groups.
1990: Chronic Toxicity/ Carcinogenicity – Rat	41643801	No treatment-related histopathological lesions were seen in the ovaries, uterus, mammary, thyroid, adrenal and pituitary glands.

Table 11. Evaluation of Data Submitted in Relation to the Uterotrophic Assay		
Chemical: Glyphosate		**PC Code: 417300**
890.1600 - Uterotrophic Assay (Rat)		
Carcinogenicity – Mouse	00130406	No treatment-related changes were seen in absolute or relative weights of the ovaries and adrenal glands. No treatment-related histopathological lesions were seen in the ovaries, uterus, mammary, thyroid, adrenal and pituitary glands.
Chronic Toxicity – Dog	00153374	No treatment-related changes were seen in absolute or relative weights of the ovaries, thyroid, adrenal and pituitary glands. No treatment-related histopathological lesions were seen in the ovaries, uterus, thyroid, adrenal and pituitary glands.
Subchronic – Rat	40559401	No treatment-related histopathological lesions were seen in the ovaries, uterus, cervix, mammary, thyroid, adrenal and pituitary glands.
Subchronic – Rat (NTP, 1992)	N/A	A significantly ($p<0.05$) longer estrous cycle length was seen in rats at the 3393 mg/kg/day (5.4 ± 0.21days) as compared to controls (4.9 ± 0.10 days). No treatment-related histopathological lesions were seen in the ovaries, uterus, vagina, clitoral glands, thyroid, adrenal and pituitary glands.
Subchronic – Mouse (NTP, 1992)	N/A	No treatment-related changes were seen in estrous cycle length at any dose level. No treatment-related histopathological lesions were seen in the ovaries, uterus, vagina, clitoral glands, thyroid, adrenal and pituitary glands.
Subchronic – Mouse	00036803	No treatment-related changes were seen in absolute or relative ovarian weights. No treatment-related histopathological lesions were seen in the ovaries, uterus, mammary, thyroid, adrenal and pituitary glands.

3. Agency's Evaluation of the OSRI:

The submission provided by the Test Order Recipient states that "Owens and Ashby (2002) conducted a thorough critical review of the uterotrophic assay, and the predictive value of the uterotrophic assay itself was determined by comparison with observations made in the multigenerational reproductive and developmental toxicity assays. They concluded that 'labeling or classification action should not be based on *in vitro* or *in vivo* screens (e.g., transcriptional assays or the uterotrophic assay), but await the results of robust, definitive testing protocols for adverse effects, such as the multigenerational reproductive and developmental assay, so that both the data on mechanism and the occurrence of an adverse effect are available.' Most importantly, this paper provided a comparison of no observed effect levels (NOEL) and lowest observed effect levels (LOEL) for uterotrophic and rat multigeneration studies, and concluded high concordance for NOEC and LOEC values for estrogenicity.

Table 11. Evaluation of Data Submitted in Relation to the Uterotrophic Assay

Chemical: Glyphosate	**PC Code: 417300**

890.1600 - Uterotrophic Assay (Rat)

There is consistent evidence showing a lack of estrogenic activity of glyphosate in four multigenerational studies, a subchronic study, and a chronic study, all in rats. These equivalent data provide strong grounds for the uterotrophic assay being considered duplicative and unnecessary to characterize glyphosate potential as a non-estrogenic compound in an *in vivo* assay."

Endpoint in Uterotrophic assay	Functionally Equivalent Information from Existing Studies
Uterine Weight	• No impact on uterine weights with cervix at the highest dose tested of 10,000 ppm in diet (Moxon, 2000) of the F0 and F1 parent animals. • No impact on uterine histopathology which is consistent with no effect on uterine weight at 10,000 ppm in diet (Moxon, 2000). • No impact on uterine weights with cervix and oviducts at the highest dose tested of 15,000 ppm in diet (Dhinsa *et al.*, 2007) of the F0 and Fl parent animals. • No impact on uterine histopathology which is consistent with no effect on uterine weight at 15,000 ppm in diet (Dhinsa *et al..*, 2007). • No impact on uterine weights at the highest dose tested of 15,000 ppm in diet (Dhinsa *et al...*, 2007) of the F1 and F2 offspring animals. • No impact on uterine histopathology which is consistent with no effect on uterine weight at 15,000 ppm in diet (Dhinsa *et al.*, 2007). • No impact on uterine weights at the highest dose tested of 15,000 ppm in diet (Dhinsa *et al...*, 2007) of the F1 and F2 offspring animals.

The submission by **PETA** states that "In a female pubertal study, an increase in estrous cycle length from 4.9 to 5.4 days was reported in the high-dose female F344 rats (3393mg/kg/day or 50,000 ppm) (NTP 1992). F344 rats, however, are known to exhibit highly variable estrous cycle lengths (4 to 6 days) leading Morrissey *et al.* (1988) to conclude that 'stages of the estrous cycle are so variable [in F344 rats] that they may not be useful in assessing potential toxicity.' Even if the estrous cycle length data were valid, they are of doubtful significance because the extremely high dosage associated with its occurrence. As no changes in sperm counts or estrous cycling were observed in mice treated at the same extremely high doses, it is concluded that glyphosate does not adversely affect sperm concentration or estrous cyclicity at any relevant dosage (NTP, 1992). It is also important to note that these dose levels are several orders of magnitude greater than any exposure ever likely to be experienced by humans."

Table 11. Evaluation of Data Submitted in Relation to the Uterotrophic Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1600 - Uterotrophic Assay (Rat)

"After reviewing the existing data for both GA and commercially available formulations, there *is no evidence that the active ingredient causes endocrine disruption.* The mechanism of glyphosate activity is not related to endocrine pathways and should not modulate any endocrine activity and experimental results support this hypothesis. Immune response depression and sperm content reduction are endpoints which could potentially be connected with endocrine effects are also not shown to be caused *in vitro* and *in vivo* by glyphosate (Takacas 2002).

No effects were observed in numerous, multigeneration reproduction studies conducted at several doses ranging from low levels to those that exceed human glyphosate exposure by several orders of magnitude. Thus, a sufficient battery of studies has been conducted to evaluate the potential for endocrine modulation. Taken together, results from all studies demonstrate that glyphosate and its primary degradate AMPA are *not reproductive toxicants* and *do not perturb the endocrine system.*

The U.S. EPA (1998a) reviewed these studies and also concluded that there was *no evidence to suggest that glyphosate produces endocrine-modulating effects*. The endocrine-modulating potential of glyphosate has been evaluated in a variety of studies including *in vitro* assays and standard *in vivo* toxicology studies.

The *in vivo* studies comprehensively assess endocrine functions that are required for reproduction, development, and chronic health. Glyphosate produced no effects in *in vitro* assays, and there was no indication of changes in endocrine function in any of the *in vivo* studies (Williams 2000).

Data is plentiful and well balanced, including many *in vitro* and *in vivo* studies, covering identical endpoints indicated in the EDSP. *No additional data is needed* to screen glyphosate active for endocrine disrupting activity because the data taken together clearly indicate no effects."

On review of the OSRI submitted, the Agency noted the following:

Although the OSRI cited for the estrogenic pathway mentions two 2-Generation Reproduction studies conducted in 2000 and 2007[890.3800 Subdivision F Guideline requirements (i.e., Post-1998)], at the time that this report was finalized these studies have not been submitted to the Agency for review. The OSRI for glyphosate also cited a 3-Generation Reproduction study conducted in 1981 and a 2-Generation Reproduction study conducted in 1990 as well as a reproductive toxicity study in mice and rats that examined sperm parameters, vaginal cytology, organ weight and histopathology of the female reproductive organs. The following results were observed:

Table 11. Evaluation of Data Submitted in Relation to the Uterotrophic Assay	
Chemical: Glyphosate	**PC Code: 417300**

890.1600 - Uterotrophic Assay (Rat)

- No treatment-related histopathological lesions were seen in the uterus of the F0 parents, F1b parents, F2b parents and F3b offspring in the 1981 3-Generation, in the F0 and F1a parents in the 1990 2-Generation Reproduction.

- The Uterotrophic Assay uses the ovariectomized (or immature) female. The uterotrophic response is an increase in uterine weight due to water imbibition. None of the cited studies measured uterine weight. The cited studies used intact animals. This difference is critical because ovariectomy removes the major endogenous source of estrogen and thus increases the sensitivity of the uterus to estrogens. As such, the Agency has remaining questions regarding the sensitivity of the uterus to estrogens in female animals exposed to the chemical.

4. Conclusion:
Based on the deficiencies discussed above, the data cited as OSRI did not satisfy the requirement for the Uterotrophic Assay using Guideline 890.1600.

[1] -- = not measured; X = indicates the endpoint was measured in the assay but does not indicate whether or not it satisfies the data requirement of the test order. See section 3 below for a detailed explanation; N/A = not applicable

III. Summary of studies cited in the OSRI submission that were considered in the EDRT's evaluation

Chemical: Glyphosate		**PC Code: 417300**
MRID No.	**Citation in OSRI**	**Selected Endocrine Related Findings**
N/A	Study Type: AR and ER Transcriptional Activation and Aromatase Assays Author: Gasnier *et al.* Year: 2005	In this study, authors performed a number a number of *in vitro* tests (including anti-E and anti-A transcriptional activation and aromatase assay) on glyphosate to evaluate cytotoxicity and its potential to affect endocrine activity. Anti-estrogencity was evaluated using HepG2 cells transiently transfected with plasmids expressing hERα, hERβ, a luciferase reporter plasmid containing ERE. Anti-androgenicity was evaluated using the human cell line-based androgen receptor gene transcription assay with MDA-MB-453-kb2 cells. Aromatase activity was measured in HepG2 cells using the tritiated water release assay. Glyphosate was shown to be negative for both anti-estrogenicity and aromatase activity and positive for anti-androgenicity in this study.
46650501	Study Type: Amphibian Study Author: Howe *et al.* Year: 2004	Howe *et al.* (2004) exposed *Rana pipiens* tadpoles to 0.6 and 1.8 mg acid equivalents/L glyphosate technical (isopropylamine salt) for 42 days (followed by rearing in clean water) from Gosner stage 25 until metamorphic climax (Gosner stage 42) and assessed mortality, snout-vent length, total length, body length, tail length, maximum tail height, visible damage to tail, sex ratio, gonadal development, and thyroid hormone receptor (TR) mRNA. Although this study assessed developmental parameters (e.g., time to metamorphosis, snout-vent length), it did not include measurements on tissues associated specifically with the thyroid axis/activity (e.g., thyroid histopathology, hind leg length) besides TR mRNA.
48033008	Study Type: AR and ER Transcriptional Activation Assays Author: Kojima *et al.* Year: 2004	In this study, Kojima *et al.*, tested 200 chemicals (including glyphosate) for agonism and antagonism to human estrogen receptor (ER α and ERβ) and human androgen receptor (AR) using Chinese Hamster Ovary-K1 cells in luciferase reporter assay. The relative activity for each pesticide tested was expressed as REC_{20} (20% relative effective concentration), which is the concentration of the test compound showing 20% of the activity of 10^{-10} M E2, 10^{-9} M E2 or 10^{-9} M DHT for ERα, ERβ or AR, respectively. Similarly, RIC_{20} (20% relative inhibitory concentration) was reported as the test material concentration showing 20% inhibition of the activity induced by 10^{-11} M E2, 10^{-10} M E2, or 10^{-10} M DHT for ERα, ERβ or AR, respectively. The authors concluded that glyphosate was negative for agonist and antagonist activity for the ER and AR.

Chemical: Glyphosate		PC Code: 417300
MRID No.	**Citation in OSRI**	**Selected Endocrine Related Findings**
	Study Type: ER Transcriptional Activation Assay Author: Petit *et al.* Year: 1997	This study evaluated glyphosate in a reporter assay using recombinant yeast cells expressing trout estrogen receptor. Glyphosate was tested at concentration range of 10^{-8} to 10^{-5} M. The authors reported that glyphosate was negative for activation of the ER in this test system.
	Study Type: Aromatase Assay Author: Richard *et al.* Year: 2005	The authors tested glyphosate and several product formulations in JEG-1 cells using radioimmunoassay (RIA) as the method of detecting reaction products. Induction of mRNA was also followed and aromatase activity was also assessed using the placental microsomal assay by measuring the formation of tritiated water. Aromatase was inhibited by glyphosate in this assay.
N/A	Study Type: Vitellogenin Assay Author: Xie *et al.* Year: 2005	This study evaluated the potential estrogenic activity of aquatic herbicides (including glyphosate) in a rainbow trout vitellogenin assay. Juvenile fish were exposed to glyphosate at a concentration of 0.11 mg/l. The authors report that after 7 days exposure, glyphosate did not induce vitellogenin levels relative to the control fish..
00081674 00105995	Study Type: Three-Generation Reproduction Classification: Minimum Year: 1981 Species: Rat Strain: Sprague-Dawley Sex: Male and Female Age at Initiation: 43 days	Dose levels tested: 0, 3, 10 and 30 mg/kg/day in the diet for three consecutive generations. The F0 parental animals were given the test diets prior to mating to produce the F1a and F1b offspring. The F1b offspring selected at weaning to be the parents were exposed to the test diet prior to mating to produce the F2a and F2b offspring. The F2b offspring selected at weaning to be parents were exposed to the test diet prior to mating to produce the F3a and F3b offspring. Exposure to the test diet continued throughout mating, gestation and lactation, with further exposure of the offspring after weaning. Reproductive toxicity: No treatment-related effects were observed on mating, pregnancy, and fertility indices or fetal sex ratio for either sex over the three generations. The statistically significant but non dose dependent decrease (83-92%) seen in the Day 4-21 pup survival at all dose levels in the F1b litters were attributed to high pup mortality within one or more litters at each dose level. Pup survival between Day 4 and 21 in the F1 and F2 generations was comparable between control and treated groups. Consequently the decreases seen in the F1b litters were not consistent and were not considered to be treatment related.

Chemical: Glyphosate		PC Code: 417300
MRID No.	**Citation in OSRI**	**Selected Endocrine Related Findings**
		Organ weights: No treatment-related changes in absolute or relative weights of the testes, ovaries, adrenal and pituitary glands of the parental (F0, F1 and F2) or the F3b offspring. Histopathology: No treatment-related histopathological lesions were seen in the testes, prostate, ovaries, uterus, mammary, thyroid, pituitary and adrenal gland of the F0, F1 and F2 adults or the F3b offspring.
41621501	Study Type: Two-Generation Reproduction Classification: Guideline Year: 1990 Species: Rat Strain: Sprague-Dawley Sex: Male and Female Age at Initiation: 7 weeks	Dose levels tested: 0, 100, 500 and 1500 mg/kg/day in the diet for two consecutive generations. The F0 animals received the test diet for 11 weeks prior to mating to produce the F1a and F1b offspring. The F1b pups selected to be parents were fed the test diets for 14 weeks prior to mating to produce the F2a and F2b litters. Exposure to the test diet continued throughout mating, gestation and lactation, with further exposure of the offspring after weaning. Reproductive toxicity: No treatment-related effects were observed in the reproductive performances (male or female mating indices, male or female fertility indices, gestation index, gestation length, live birth index, viability index, lactation index, parturition, or fetal sex ratio). Organ weights: No treatment-related changes in absolute or relative weights of the testes or ovaries were seen in the F0 or the F1 adults. Histopathology: No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicle, prostate, ovaries, uterus, vagina, mammary, or pituitary (F1 only) gland of the F0 and F1 adults.
00046362	Study Type: Developmental Toxicity Classification: Guideline Year: 1980 Species: Rat	Dose levels tested: 0, 300, 1000 and 3500 mg/kg/day in 0.5% aqueous methocel via gavage on days 6 through 15 of gestation; dams were sacrificed on gestation day 20. No treatment-related changes were seen in the number of corpora lutea, resorptions, fetal sex ratio, or soft tissue abnormalities at any dose. Viable fetuses/dam were significantly ($p<0.05$) decreased to 11.9 ± 4.36 and 11.5 ± 4.12 at 300 and 3500 mg/kg/day, respectively compared to the controls (14.4 ± 1.26). Total implantations/dam were decreased to 12.1 ± 4.45 ($p<0.001$) at 300 mg/kg/day and 12.8 ± 3.77 at 3500 mg/kg/day, respectively compared to the controls (15.0

Chemical: Glyphosate		PC Code: 417300
MRID No.	**Citation in OSRI**	**Selected Endocrine Related Findings**
	Strain: Sprague-Dawley Sex: Pregnant female Age at Initiation: 14 weeks	± 1.67).
00046363	Study Type: Developmental Toxicity Classification: Guideline Year: 1980 Species: Rabbit Strain: Dutch Belted Sex: Pregnant female Age at Initiation: 7 months	Dose levels tested: 0, 75, 175 and 350 mg/kg/day in 0.5% aqueous methocel via gavage on days 6 through 27 of gestation; does were sacrificed on gestation day 28. No treatment-related changes were seen in the number of corpora lutea, implantation sites, early or late resorptions, number of live fetuses/litter, fetal sex ratio, or soft tissue abnormalities at any dose.
00093879 00150564	Study Type: Chronic Toxicity/ Carcinogenicity Classification: Minimum Year: 1981 Species: Rat Strain: Sprague-Dawley	Dose levels tested: 0, 3.05, 10.30 and 31.49 mg/kg/day for males and 0, 3.37, 11.22 and 34.02 mg/kg/day for females in the diet for 26 months Organ weights: No treatment-related changes were seen in absolute or relative weights of the testes, ovaries, thyroid, pituitary and adrenal glands. Histopathology: No treatment-related histopathological changes were seen in the epididymides, seminal vesicles, prostate, ovaries, uterus, mammary glands, adrenal and pituitary glands. Testes: Testicular interstitial cell tumors were seen in 3/50, 1/50 and 6/50 at the low, mid and high dose groups when compared to controls (0/50) with the 12% incidence at the high dose

Chemical: Glyphosate		PC Code: 417300
MRID No.	**Citation in OSRI**	**Selected Endocrine Related Findings**
	Sex: Male and Female Age at Initiation: Not reported	reaching statistical significance (p=0.013) and were markedly higher than the overall historical control incidence of 4.5% (24/535 animals) Thyroid: C-cell carcinomas were seen in 5/47 (11%; p<0.001) females at the high dose when compared to control females (1/47; 2%). No increases were seen in adenomas and the incidences of C-cell hyperplasia were comparable between the treated and the control groups.
41643801	Study Type: Chronic Toxicity/ Carcinogenicity Classification: Guideline Year: 1990 Species: Rat Strain: Sprague-Dawley Sex: Male and Female Age at Initiation: 8 weeks	Dose levels tested: 0, 89, 362 and 940 mg/kg/day for males and 0, 113, 457 and 1183 mg/kg/day for females in the diet for 104 weeks. Organ weights: No treatment-related changes were seen in absolute or relative weights of the testes, epididymides and prostate glands. Histopathology: No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicle, prostate, ovaries, uterus, and mammary, adrenal and pituitary glands. The incidences of thyroid C-cell adenomas were increased in males at the mid (8/58; 13.8%) and high dose (7/60; 11.7%) groups and in females at the mid (6/60; 10%) and high (6/60; 10%) dose groups compared to control males (2/60; 3.3%) and females (2/60; 3.3%). These increases were not considered to be treatment-related because: 1) none of the increases reached statistical significance; 2) absence of dose-response; 3) no increase in severity of grade or incidence in hyperplasia; 4) lack of progression to malignancy; and 4) the incidences were within the testing laboratories historical control ranges.
00093879	Study Type: Carcinogenicity Classification: Minimum Year: 1983 Species: Mouse Strain: CD-1	Dose levels tested: 0, 20, 100 and 600 mg/kg/day in the diet for 24 months. Organ weights: No treatment-related changes were seen in absolute or relative weights of the testes, ovaries and adrenal glands. Histopathology: No treatment-related histopathological lesions were seen in the testes (with epididymides), prostate, ovaries, uterus, mammary, thyroid, adrenal and pituitary glands.

Chemical: Glyphosate		PC Code: 417300
MRID No.	**Citation in OSRI**	**Selected Endocrine Related Findings**
	Sex: Male and Female Age at Initiation: 5.5 weeks	
00153374	Study Type: Chronic Toxicity Classification: Guideline Year: 1985 Species: Dog Strain: Beagle Sex: Male and Female Age at Initiation: Not reported	Dose levels tested: 0, 20, 100 and 500 mg/kg/day in the diet for 1 year. Organ weights: No treatment-related changes were seen in absolute or relative weights of the testes, ovaries, thyroid, adrenal and pituitary glands. Histopathology: No treatment-related histopathological lesions were seen in the testes, ovaries, uterus, thyroid, adrenal and pituitary glands.
40559401	Study Type: Subchronic Toxicity Classification: Guideline Year: 1987 Species: Rat Strain: Sprague-Dawley Sex: Male and Female Age at Initiation: 6 weeks	Dose levels tested: 0, 63, 317 and 1267 mg/kg/day for males and 0, 84, 404 and 1623 mg/kg/day for females in the diet for 90 days. Organ weights: No treatment-related changes were seen in absolute or relative weights of the testes (with epididymides). Histopathology: No treatment-related histopathological lesions were seen in the testes (with epididymides), seminal vesicle, prostate, ovaries, uterus (corpus and cervix), mammary, thyroid, adrenal and pituitary glands

Chemical: Glyphosate		PC Code: 417300
MRID No.	**Citation in OSRI**	**Selected Endocrine Related Findings**
NTP (1992)	Study Type: Subchronic Toxicity Classification: Not applicable Year: 1992 Species: Rat Strain: F/344 Sex: Male and Female Age at Initiation: 43 days	Dose levels tested: 0, 205, 410, 811, 1678, 3393 mg/kg/day for males and 0, 213, 421, 844, 1690 and 3393 mg/kg/day for females in the diet for 90 days. Sperm parameters: Sperm counts were significantly ($p < 0.01$) decreased (20%) at 1678 and 3393 mg/kg/day dose groups. Epididymal sperm motility, total spermatid head/testes, and total spermatid heads/g caudal tissue in the treated animals were similar to the controls. Estrous cycle: A significantly ($p<0.05$) longer estrous cycle length was seen in rats at the 3393 mg/kg/day (5.4 ± 0.21days) as compared to controls (4.9 ± 0.10 days). Organ weights: No treatment-related changes were seen in left caudal, epididymal or testicular weights. Relative weights of the right testes were significantly increased at 1678 mg/kg/day dose (7%; $p < 0.05$) and at 3393 mg/kg/day dose (21%; $p < 0.01$) groups. Absolute weights in treated rats were comparable to the controls. Histopathology: No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicles, prostate, preputial gland, ovaries, uterus, vagina, clitoral glands, thyroid, adrenal and pituitary glands.
NTP (1992)	Study Type: Subchronic Toxicity Classification: Not applicable Year: 1992 Species: Mice Strain: B6C3F1 Sex: Male and Female Age at Initiation: 49 days	Dose levels tested: 0, 507, 1065, 2273, 4776 and 10780 mg/kg/day for males and 0,753, 1411, 2707, 5846 and 11977 mg/kg/day for females in the diet for 90 days. Sperm parameters: No treatment-related changes were seen in sperm concentration, motility, counts or morphology at any dose level. Estrous cycle: Not treatment-related changes were seen in estrous cycle length at any dose level. Organ weights: No treatment-related changes were seen in left caudal, epididymal and testicular weights. Relative weights of the right testes were significantly ($p < 0.01$) increased at 4776 mg/kg/day dose (10%) and at 10,780 mg/kg/day dose (18%) groups. Absolute weights in treated rats were comparable to the controls.

Chemical: Glyphosate		PC Code: 417300
MRID No.	**Citation in OSRI**	**Selected Endocrine Related Findings**
		Histopathology: No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicles, prostate, preputial gland, ovaries, uterus, vagina, clitoral glands, thyroid, adrenal and pituitary glands.
00036803	Study Type: Subchronic Toxicity Classification: Minimum Year: 1979 Species: Mice Strain: CD Sex: Male and Female Age at Initiation: 6 weeks	Dose levels tested: 0, 250, 500 and 2500 mg/kg/day in the diet for 90 days. Organ weights: No treatment-related changes were seen in absolute or relative weights of the testes and ovaries. Histopathology: No treatment-related histopathological lesions were seen in the testes, epididymides, prostate, ovaries, uterus, mammary, thyroid, adrenal and pituitary glands.
00111953	Study Type: Avian Reproduction Classification: Acceptable Year: 1978 Species: Mallard duck (*Anas platyrhynchos*) Sex: Males and females Controls: In corn oil	Exposure concentrations: 0 (control), 50, 200, and 1000 ppm a.i., (nominal concentrations, 83% TGAI) Reproductive Parameters: No treatment-related effects on reproduction were seen up to the highest treatment level, 1000 ppm. Survival: No treatment related effects on survival were seen up to the highest treatment level, 1000 ppm. Endpoints such as survival and growth (*i.e.*, length and weight) that were measured in the study are not directly related to reproduction but may be affected by endocrine-mediated processes. Overall: NOAEC = 1000 ppm, LOAEC > 1000 ppm

Chemical: Glyphosate		PC Code: 417300
MRID No.	**Citation in OSRI**	**Selected Endocrine Related Findings**
	Study Type: Avian Reproduction Classification: Acceptable Year: 1978 Species: Bobwhite quail (*Colinus virginianus*) Sex: Males and females Controls: In corn oil.	Exposure concentrations: 0 (control), 50, 200, and 1000 ppm a.i., (nominal concentrations, 83% TGAI) Reproductive Parameters: • No treatment related effects on reproduction were seen up to and including the highest treatment level, 1000 ppm. Survival: • No treatment related effects on survival were seen up to and including the highest treatment level, 1000 ppm. Growth (*i.e.* length & weight): No treatment related effects on survival were seen up to and including the highest treatment level, 1000 ppm. Overall: NOAEC < 1000 ppm, LOAEC = 1000 ppm.
00108171	Study Type: Fish Full Life Cycle Classification: Acceptable Year: 1975 Species: Fathead Minnow (*Pimephales Promelas)* Sex: Males and females Age: eggs	Exposure concentrations: 0 (control), 1.6, 3.2, 6.3, 12.5, 25 mg/L Reproductive Parameters: egg production of first generation fish, and on hatchability of second generation eggs and fry. No treatment related effects on reproduction were seen up to and including the highest treatment level, 25 mg/L Survival: No treatment related effects on survival were seen up to and including the highest treatment level, 25 mg/L. Growth (i.e. length & weight): No treatment related effects on growth were seen up to and including the highest treatment level, 25 mg/L. Overall: NOAEC 25 mg/L, LOAEC >= 25 mg/L.

IV. Studies cited in the OSRI but were not used in the EDRT's weight of evidence evaluations.

Chemical: GLYPHOSATE		**PC Code: 417300**
MRID No.	**Study Type or Literature Citation**	**Reasons for not using the study**
00108171	The acute toxicity portion of: EG & G, Bionomics. (1975). Chronic Toxicity of Glyphosate to the Fathead Minnow (Pimephalespromelas, Rafmesque). (Unpublished study received Dec 27, 1978 under 524-308; submitted by Monsanto Co., Washington, DC; CDL:097759-B).	The acute toxicity portion of this study was cited as OSRI as well as the chronic portion of this study. The chronic portion was assessed in the EDRT weight of the evidence evaluation. The acute toxicity data cited were related to acute toxicity classification and had no endocrine-related references.
N/A	Benachour *et al.* (2007)	This study tested a formulated product, Bioforce ®
N/A	Benachour and Seralini (2009)	This study tested Roundup ® formulations.
00067039	Birch, M.D. (1970) Toxicological Investigation of CP 67573-3: Project No. Y-70-90. (Unpublished study received Jan 30, 1973 under 524-308; prepared by Younger Laboratories, Inc., submitted by Monsanto Co., Washington, D.C.; CDL:008460-C).	This citation contains results of acute toxicity studies.
00108099	Colvin, L.; Miller, J.; Marvel, J. (1973) Final Report on CP 67573 Residue and Metabolism: Part 9: The Gross DistributionCP 67573-14C (N-phosphono-methylglycine-14C) in the Rabbit: Agricultural Research Report No. 298. (Unpublished study received Nov 12, 1973 under 4G1444; submitted by Monsanto Commercial Products Co., St. Louis, MO; CDL: 093849-D)	This metabolism study did not evaluate parameters related to endocrine toxicity/disruption.
N/A	Dallegrave *et al.* (2003)	This study tested Roundup ® formulation of Brazil.
N/A	Dallegrave *et al.* (2007)	This study tested Roundup ® formulation of Brazil.
N/A	Degitz SJ, GW Holcombe, KM Flynn, PA Kosian, JJ Korte, JE Tietge. (2005). Progress towards development of an amphibian-based thyroid screening assay using Xenopus laevis. Organismal and thyroidal responses to the model compounds 6-propylthiouracil, methimazole, and thyroxine. Toxicol Sci. 87:353-64.	Glyphosate was not tested in this study.

Chemical: GLYPHOSATE		PC Code: 417300
MRID No.	**Study Type or Literature Citation**	**Reasons for not using the study**
N/A	Dhinsa *et al.* (2007); Two-Generation Reproduction	Study cited in OSRI but not submitted to the Agency.
N/A	ECETOC Technical Report, No. 106. Guidance on Identifying Endocrine Disrupting Effects. http://www.ecetoc.org/technical-reports. Available upon request.	This is a report, not a study.
N/A	Evans, D.D. and Marian J. Batty. (1986). Effects of high dietary concentrations of glyphosate (round-up) on a species of bird, marsupial, and rodent indigenous to Australia. Environmental Toxicology and Chemistry 5 (4): 399-401.	This study was conducted with a formulation of glyphosate which contains inerts. The potential activity of the inerts on the endocrine system may confound the effects. Therefore, this study cannot be considered as part of the weight of the evidence evaluation.
00076492	Fink, R. (1973a) Final Report: Eight-day Dietary LC50—Bobwhite Quail: Project No. 241-106 (Unpublished study received Nov 9, 1973 under 524-308; prepared by Environmental Sciences Corp., submitted by Monsanto Co., Washington, D.C.; CDL:120640-D).	This is a 5-day subacute dietary study on immature birds. There were no references to endocrine-related effects.
00108107	Fink, R. (1973) Final Report: Eight-day Dietary LC50--Mallard Ducks: Technical CP67573|: Project No. 241-107. (Unpublished study received Jul 12, 1974 under 5F1536; prepared by Environmental Sciences Corp., submitted by Monsanto Co., Washington, DC; CDL:094171-I).	The OSRI states that "reproductive impairment (unspecified) was seen at levels above 4640 ppm". The Agency did not find any reference to reproductive impairment. Note: this is a 5-day study on 14-day old birds.
00162296	Folmar, L.; Sanders, H.; Julin, A. (1979) Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Archives of Environmental Contaminants and Toxicology 8:269-278.	In the OSRI document, the data cited were related to acute toxicity classification and had no endocrine-related references.
N/A	Fort DJ, S Degitz, J Tietge, and LW Touart. (2007). The hypothalamic-pituitary-thyroid (HPT) axis in frogs and its role in frog development and reproduction. Crit Rev Toxicol. 37:117-161.	This is a report, not a study.
40767102	Howe, R.; Chott, R.; McClanahan, R. (1988) Metabolism of Glyphosate in Sprague-Dawley Rats. Part II. Identification, Characterization, and Quantitation of Glyphosate and Its Metabolites after Intravenous and Oral Administration:	This metabolism study did not evaluate parameters related to endocrine toxicity/disruption.

Chemical: GLYPHOSATE		PC Code: 417300
MRID No.	**Study Type or Literature Citation**	**Reasons for not using the study**
	Laboratory Project No. MSL-7206: R.D. No. 877. Unpublished study prepared by Monsanto Co. 155 p.	
N/A	Mann, R.M. and J.R. Bidwell. (1999). The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs. Archives of Environmental Contaminants and Toxicology, 36:193-199.	This study was not specifically cited; however, it was in the bibliography of one of the OSRI documents. This is an acute toxicity study on several species of frogs. There are no endocrine-related references in the study.
00108205	McAllister, W.; Forbis, A. (1978) Acute Toxicity of Technical Glyphosate to Bluegill Sunfish (Lepomis macrochirus): Static Acute Bioassay Report. (Unpublished study received Jul 14, 1978 under 524-308; prepared by Analytical Bio Chemistry Laborato- ries, Inc., submitted by Monsanto Co., Washington, DC; CDL: 234395-B). MRID 00108205.	In the OSRI document, the data cited were related to acute toxicity classification and had no endocrine-related references.
N/A	Morrissey *et al.* (1988)	The authors reported the results of analyses of the sperm morphology and vaginal cytology examinations conducted in the subchronic studies conducted by the National Toxicology Program from 1983 to 1986. Data specific to glyphosate was not reported in this article.
N/A	Moxon 2000; Two-Generation Reproduction	Study cited in OSRI but not submitted to the Agency.
N/A	Oliveira AG, Telles LF, Hess RA, Mahecha GA, Oliveira CA. (2007). Effects of the herbicide Roundup on the epididymal region of drakes Anas platyrhynchos. Reproductive Toxicology 23(2):182-91.	This study was not specifically cited; however, it was in the bibliography of one of the OSRI documents. The study was conducted with a formulation of glyphosate which contains inerts. The potential activity of the inerts on the endocrine system may confound the effects. Therefore, this study cannot be considered as part of the weight of the evidence evaluation.
N/A	Opitz R, I Lutz, NH Nguyen, TS Scanlan, W Kloas. (2006). Analysis of thyroid hormone receptor betaA mRNA expression in Xenopus laevis tadpoles as a means to detect agonism and antagonism of thyroid hormone action. Toxicol Appl Pharmacol. 212:1-13.	Glyphosate was not tested in this study.

Chemical: GLYPHOSATE		PC Code: 417300
MRID No.	**Study Type or Literature Citation**	**Reasons for not using the study**
N/A	Opitz R, T Braunbeck, C Bogi, DB Pickford, G Nentwig, J Oehlmann, O Tool, I Lutz, W Kloas. (2005). Description and initial evaluation of a Xenopus metamorphosis assay for detection of thyroid system-disrupting activities of environmental compounds. Environ Toxicol Chem. 24:653-64.	Glyphosate was not tested in this study.
00132685	Ridley, W, Dietrich, M.; Folk, R.; *et al...* (1983) A Study of the Plasma and Bone Marrow Levels of Glyphosate following Intraperitoneal Administration in the Rat: Study No. 830109. (Unpublished study received Nov 15, 1983 under 524-308; submitted by Monsanto Co., Washington, DC; CDL:251737-F)	This metabolism study did not evaluate parameters related to endocrine toxicity/disruption.
40767101	Ridley, W.; Mirly, K. (1988) The Metabolism of Glyphosate in Sprague Dawley Rats—Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites following Intravenous and Oral Administration: Laboratory Project No. 86139 (MSL-7215): R.D.No. 877. Unpublished study prepared by Monsanto Co. 587 p.	This metabolism study did not evaluate parameters related to endocrine toxicity/disruption.
N/A	Romano *et al.* (2010)	This study tested Roundup Transorb ® formulation.
N/A	SERA (Syracuse Environmental Research Associates). 2002. Neurotoxicity, Immunotoxicity, and Endocrine Disruption with Specific Commentary on Glyphosate, Triclopyr, and Hexazinone: Final Report, Prepared for the USDA, forest service by Patrick Durkin and Gary Diamond. Final Report Syracuse Environmental Research Associates, Inc. USDA Order No. 43-3187-2-6002	This is a report, not a study.
N/A	Sumpter JP and S Jobling. (1995). Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environ Health Perspect. 103 Suppl 7:173-8.	This is a review article.
N/A	Takacs *et al.* (2002)	This is technical report that assessed the potential toxicity of agricultural products, including glyphosate, to wildlife. Not glyphosate-specific data. What was summarized from this report were acute toxicity data (e.g., LD_{50} values) and had no endocrine-related references.

Chemical: GLYPHOSATE		PC Code: 417300
MRID No.	**Study Type or Literature Citation**	**Reasons for not using the study**
N/A	Tietge JE, GW Holcombe, KM Flynn, PA Kosian, JJ Korte, LE Anderson, DC Wolf, SJ Degitz. (2005). Metamorphic inhibition of Xenopus laevis by sodium perchlorate: effects on development and thyroid histology. Environ Toxicol Chem. 24:926-33.	Glyphosate was not tested in this study.
N/A	Trotter, *et al.* (1990).	This is technical report (Canadian water quality guidelines). There were only summary data for glyphosate that included acute toxicity data (e.g., LD_{50} and LC_{50} values) and had no endocrine-related references.
N/A	USEPA (1998a)	This is a Federal Register Notice.
N/A	Walsh *et al.* (2000)	This study tested Roundup ® formulations.
N/A	Williams *et al.* (2000)	This is a review article on the "Safety Evaluation and Risk Assessment of the Herbicide Roundup and Its Active Ingredient, Glyphosate, for Humans". The data presented in this review articles are included in EDRT's evaluation.

V. Bibliography of Existing Data Cited in the OSRI

(i) Part 158 Studies

MRID	Citation
00036803	Street, R.W.; Conkin, R.A.; Edwards, G.A.; *et al...* (1980) A Three-Month Feeding Study of Glyphosate in Mice: Special Report# MSL-1154. (Unpublished study received Jul 2, 1980 under 524308; submitted by Monsanto Co., Washington, D.C.; CDL:242799.
00046362	Rodwell, DE, EJ Tasker, AM Blair *et al..* (1980a) Teratology Study in Rats: IRDC No. 401-054, Study No. IR 79-016. (Unpublished study including IRDC no. 999-021; received May 23, 1980 under 524-308; prepared by International Research and Development Corp., submitted by Monsanto Co., Washington, D.C.; CDL:242516-A.
00046363	Rodwell, DE, EJ Tasker, AM Blair *et al...* (1980b) Teratology Study in Rabbits: IRDC No. 401056, Study No. IR 79-018. (Unpublished study; prepared by International Research and Development Corp., submitted by Monsanto Co., Washington, D.C.; CDL: 242516-B).
00067039.	Birch, M.D. (1970) Toxicological Investigation of CP 67573-3: Project No. Y-70-90. (Unpublished study received Jan 30, 1973 under 524-308; prepared by Younger Laboratories, Inc., submitted by Monsanto Co., Washington, D.C.; CDL:008460-C).
00076492	Fink, R. (1973a) Final Report: Eight-day Dietary LC50—Bobwhite Quail: Project No. 241-106 (Unpublished study received Nov 9, 1973 under 524-308; prepared by Environmental Sciences Corp., submitted by Monsanto Co., Washington, D.C.; CDL:120640-D).
00081674	Schroeder RE and GK Hogan. (1981) A Three-Generation Reproduction Study with Glyphosate in Rats: Project No. 77-2, Study No. BDN 77-417 (Unpublished study received Sep 22,1981 under 524-308; prepared by Bio/dynamics, Inc., submitted by Monsanto Co., Washington, D.C.; CDL: 245909-A).
00093879	Lankas, G.R.; Hogan, G.K. (1981) A Lifetime Feeding Study of Glyphosate (Roundup Technical) in Rats: Project No. 772062. (Unpublished study received Jan 20, 1982 under 524-308; prepared by Bio/dynamics, Inc., submitted by Monsanto Co., Washington, D.C.; CDL:246617-A; 246618; 246619; 246620; 246621).
00105995	Street, R. (1982) Letter sent to R. Taylor dated Jul 6, 1982: Roundup Herbicide: Addendum to pathology report for a three-generation reproduction study in rats with glyphosate. (Unpublished study received Jul 7, 1982 under 524-308; submitted by Monsanto Co., Washington, DC; CDL:247793-A).

00108099 Colvin L, J Miller, J. Marvel. (1973). Final Report on CP 67573 Residue and Metabolism: Part 9: The Gross Distribution CP 67573-14C (N-phosphoncmethylglycine-^{14}C) in the Rabbit: Agricultural Research Report No. 298. (Unpublished study received Nov 12, 1973 under *4G1444;* submitted by Monsanto Commercial Products Co., St. Louis, MO; CDL: 093849-D).

00108107 Fink, R. (1973b) Final Report: Eight-day Dietary LC50--Mallard Ducks: Technical CP67573: Project No.241-107. (Unpublished study received Jul 12, 1974 under 5F1536; prepared by Environmental Sciences Corp., submitted by Monsanto Co., Washington ,DC; CDL:094171-I).

00108171 EG & G, Bionomics. (1975). Chronic Toxicity of Glyphosate to the Fathead Minnow *(Pimephales promelas,* Rafinesque). (Unpublished study received Dec 27, 1978 under 524-308; submitted by Monsanto Co., Washington, DC; CDL:097759-B).

00108204 Fink, R.; Beavers, J.; Brown, R. (1978) Final Report: Acute Oral LD50--Bobwhite Quail: Technical Glyphosate: Project No. 139140.(Unpublished study received Jul 14, 1978 under 524-308; prepared by Wildlife International, Ltd. and Washington College, submitted by Monsanto Co., Washington, DC; CDL:234395-A).

00108205 McAllister, W.; Forbis, A. (1978) Acute Toxicity of Technical Glyphosate to Bluegill Sunfish: Static Acute Bioassay Report. (Unpublished study received Jul 14, 1978 under 524-308; prepared by Analytical Bio Chemistry Laboratories, Inc., submitted by Monsanto Co., Washington, DC; CDL:234395-B).

00108207 Fink, R and Beavers, J. (1978) Final Report: One-generation Reproduction study-Bobwhite Quail: Glyphosate Technical: Project No. 139-141. (Unpublished study received Nov 13, 1978 under 524-308; prepared by Wildlife International, Ltd., submitted by Monsanto Co., Washington, DC; CDL: 235924-B).

00111953 Fink, R. and Beavers, J. (1978) Final Report: One-generation Reproduction Study-Mallard Duck: Glyphosate Technical: Project No. 139-143. (Unpublished study received Nov 13, 1978 under 524-308; prepared by Wildlife International Ltd., submitted by Monsanto Co., Washington, DC; CDL:235924-A).

00130406 Knezevich, A.; Hogan, G. (1983) A Chronic Feeding Study of Glyphosate (Roundup Technical) in Mice: Project No. 77-2061: BDN-77420. Final rept. (Unpublished study received Aug 17, 1983 under 524-308; prepared by Bio/dynamics, Inc., submitted by Monsanto Co., Washington, DC; CDL:251007-A; 251008; 251009; 251010; 251011; 251012; 251013; 251014)

00132685 Ridley W, M Dietrich, and R Folk. (1983). A Study of the Plasma and Bone Marrow Levels of Glyphosate following Intraperitoneal Administration in the Rat: Study No. 830109. (Unpublished study received Nov 15, 1983 under 524-308; submitted by Monsanto Co., Washington, DC; CDL:251737-F).

00150564 McConnel, R. (1985) A Chronic Feeding Study of Glyphosate (Roundup Technical in Mice): Pathology Report on Additional Kidney Sections: Addendum to Final Report Dated July 21, 1983: Project No. 77-2061A. Unpublished study prepared by Bio/dynamics Inc. 59 p.

00153374 Reyna, M. (1985) Twelve Month Study of Glyphosate Administered by Gelatin Capsule to Beagle Dogs: Project No. ML-83-137: Study No. 830116. Unpublished study prepared by Monsanto Company Environmental Health. 317 p.

40559401 Stout L and C Johnson. (1987) 90-day Study of Glyphosate Administered in Feed to Sprague Dawley Rats: Proj. ID ML-86-351/EHL 86128. Unpublished study prepared by Monsanto Agricultural Co.

40767101 Ridley. W. and Mirly, K. (1988). The Metabolism of Glyphosate in Sprague Dawley Rats--Part I. Excretion and Tissue Distribution of Glyphosate and Its Metabolites following Intravenous and Oral Administration: Laboratory Project No. 86139 (MSL-7215): R.D. No. 877. Unpublished study prepared by Monsanto Co.

40767102 Howe, R, Chott,.R and McClanahan, R. (1988). Metabolism of Glyphosate in Sprague-Dawley Rats. Part II. Identification, Characterization, and Quantitation of Glyphosate and Its Metabolites after Intravenous and Oral Administration: Laboratory Project No. MSL-7206: R.D. No. 877. Unpublished study prepared by Monsanto Co.

41228301 Forbis A. (1989). Uptake, Depuration and bioconcentration of Carbon 14-Glyphosate to Bluegill Sunfish *(Lepomis macrochirus):* Project ID MSL-9304. Unpublished study prepared by Analytical Biochemistry Laboratories, Inc. 425 p.

41621501 Reyna M. (1990) Two Generation Reproduction Feeding Study with Glyphosate in Sprague Dawley Rats: Lab Project No: MSL-I0387, Study No. ML 88-106. Unpublished study prepared by Monsanto Agricultural Co.

41643801 Stout, L. and Ruecker, F. (1990) Chronic Study of Glyphosate administered in Feed to Albino Rats: Lab Project Number: MSL-I0495: R.D. 1014, Study No. ML 87-148. Unpublished study prepared by Monsanto Agricultural Co.

The following Test Order Recipient OSRI citations are for studies that were not submitted to the Agency at the time of review and reporting. Therefore, they were not considered in this evaluation.

Moxon, ME. (2000) Glyphosate acid: multigenerational reproduction toxicity study in rats. Unpublished study to be submitted to EPA by Syngenta Crop Protection, Inc.

Dhinsa, N. K, Watson, P. and Brooks, N.P. (2007). Glyphosate Technical: Dietary Two Generation Reproduction Study in the Rat. Unpublished study to be submitted to EPA by Nufarm Americas.

ii. Literature Citations

Benachour N, Sipahutar H, Moslemi S, Gasnier C, Travert C, Séralini GE. (2007). Time- and dose dependent effects of roundup on human embryonic and placental cells. Archives of Environmental Contaminants and Toxicology 53(1):126-33.

Benachour N, and Séralini GE. (2009). Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. Chemical Research in Toxicology 22(1):97-105.

Dallegrave E, Mantese FD, Coelho RS, Pereira JD, Dalsenter PR, Langeloh A. (2003). The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats. Toxicology Letters 142(1-2):45-52.

Dallegrave E, Mantese FD, Oliveira RT, Andrade AJ, Dalsenter PR, Langeloh A. (2007). Pre- and postnatal toxicity of the commercial glyphosate formulation in Wistar rats. Archives of Toxicology 81(9): 665-73.

Degitz SJ, GW Holcombe, KM Flynn, PA Kosian, JJ Korte, JE Tietge. (2005). Progress towards development of an amphibian-based thyroid screening assay using *Xenopus laevis.* Organismal and thyroidal responses to the model compounds 6-propylthiouracil, methimazole, and thyroxine. Toxicol Sci. 87:353-64.

Evans, D.D. and Marian J. Batty. (1986). Effects of high dietary concentrations of glyphosate (round-up) on a species of bird, marsupial, and rodent indigenous to Australia. Environmental Toxicology and Chemistry 5 (4): 399-401.

Folmar, L.; Sanders, H.; Julin, A. (1979) Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. *Archives of Environmental Contaminants and Toxicology* 8:269-278.

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